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(71) Applicant *(for all designated States except US)*: THE DOW CHEMICAL COMPANY [US/US]; 2030 Dow Center, Midland, MI 48674 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: ANDERSON, William, Henry, Kerr [US/US]; 5112 Cortland Street, Midland, MI 48642 (US). TEMPEST, Philip, R. [GB/GB]; 43 High Street, West Wratting, Cambridge CB1 5LU (GB). CARR, Frank, J. [GB/GB]; Birchlea, The Holdings, Balmedie AB23 8XR (GB). HARRIS, William, J. [GB/GB]; 18 Queen Street, Carnoustie, Angus DD7 7AB (GB). ARMOUR, Kathryn [GB/GB]; 43 High Street, West Wratting, Cambridge CB1 5LU (GB).

(74) Agent: KIMBLE, Karen, L.; Patent Dept., P.O. Box 1967, Midland, MI 48641-1967 (US).

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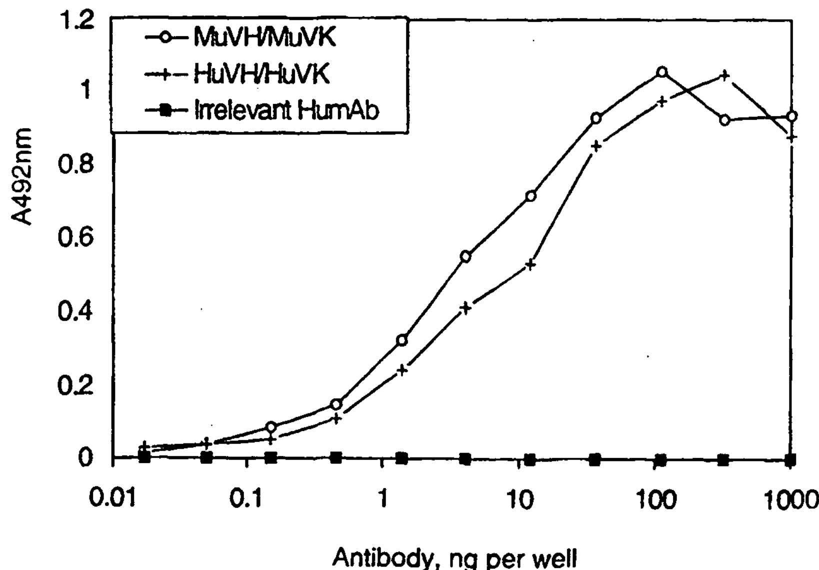

(57) Abstract

Novel humanized monoclonal antibodies, humanized antibody fragments, and derivatives thereof which specifically bind TAG-72 are provided as well as methods for their manufacture. These humanized antibodies are useful in the treatment of cancers which express TAG-72 as well as for diagnostic purposes, *e.g.*, for *in vivo* imaging of tumors or cancer cells which express TAG-72.

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HIGH AFFINITY HUMANIZED ANTI-TAG-72 MONOCLONAL ANTIBODIES

FIELD OF THE INVENTION

5 The present invention relates to humanized monoclonal antibodies and fragments or derivatives thereof which specifically bind tumor-associated glycoprotein TAG-72, a human pancarcinoma antigen expressed by various human tumor cells. More specifically, the present invention relates to humanized monoclonal antibodies and fragments or derivatives thereof derived from murine monoclonal antibody CC49 or other murine antibodies which 10 specifically bind TAG-72. The present invention further relates to methods for producing such humanized monoclonal antibodies specific to TAG-72, pharmaceutical and diagnostic compositions containing such humanized monoclonal antibodies, and methods of use thereof for the treatment or diagnosis of cancer.

BACKGROUND OF THE INVENTION

15 The identification of antigens expressed by tumor cells and the preparation of monoclonal antibodies which specifically bind such antigens is well known in the art. Anti-tumor monoclonal antibodies exhibit potential application as both therapeutic and diagnostic agents. Such monoclonal antibodies have potential application as diagnostic agents because they specifically bind tumor antigens and thereby can detect the presence of tumor cells or 20 tumor antigen in an analyte. For example, use of monoclonal antibodies which bind tumor antigens for *in vitro* and *in vivo* imaging of tumor cells or tumors using a labeled form of such a monoclonal antibody is conventional in the art.

Moreover, monoclonal antibodies which bind tumor antigens have well known application as therapeutic agents. The usage of monoclonal antibodies themselves as 25 therapeutic agents, or as conjugates wherein the monoclonal antibody is directly or indirectly attached to an effector moiety, *e.g.*, a drug, cytokine, cytotoxin, etc., is well known.

Essentially, if the monoclonal antibody is attached to an effector moiety the monoclonal antibody functions as a targeting moiety, *i.e.* it directs the desired effector moiety (which typically possesses therapeutic activity) against a desired target, *e.g.*, a tumor which 30 expresses the antigen bound by the monoclonal antibody. In contrast, when the monoclonal antibody itself operates as a therapeutic agent, the antibody functions both as a targeting moiety -- *i.e.*, it will specifically bind a cell which expresses the antigen -- and as an effector

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which mediates therapeutic activity, typically tumor cell lysis. Such effector functions -- including, *e.g.*, antibody-dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC), among others -- are effected by the portion of the antibody molecule generally referred to in the literature as the Fc portion. One specific tumor antigen against
5 which various monoclonal antibodies have been developed is tumor-associated glycoprotein TAG-72. TAG-72 is expressed on the surface of various human tumor cells, such as the LS174T tumor cell line (American Type Tissue Collection (ATCC) No. CL188, a variant of cell line LS180 (ATCC No. CL187)), a colon adenocarcinoma line. Various research groups have reported the production of monoclonal antibodies to TAG-72. See, *e.g.*, Muraro *et al.*,
10 *Cancer Res.*, 48:4588-4596 (1988); Johnson *et al.*, *Cancer Res.*, 46:850-857 (1986); Molinolo *et al.*, *Cancer Res.*, 50:1291-1298 (1990); Thor *et al.*, *Cancer Res.*, 46:3118-3127 (1986); EP 394277 to Schlom *et al.* (assigned to the National Cancer Institute); and U.S. Patent 5,512,443 to Jeffrey Schlom *et al.* Specific antibodies to TAG-72 which are publicly available include CC49 (ATCC No. HB 9459), CC83 (ATCC No. HB 9453), CC46 (ATCC No.
15 HB 9458), CC92 (ATCC No. HB 9454), CC30 (ATCC No. HB 9457), CC11 (ATCC No. 9455), and CC15 (ATCC No. HB 9460).

One example thereof, CC49, is a murine monoclonal antibody of the IgG_1 isotype. This monoclonal antibody is a second generation monoclonal antibody prepared by immunizing mice with TAG-72 purified using the first generation antibody B72.3. Colcher *et*
20 *al.*, *Proc. Natl. Acad. Sci. USA*, 78:3199-3203 (1981). CC49 specifically binds TAG-72, and has a higher antigen-binding affinity than B72.3. Muraro *et al.*, *Cancer Res.*, 48:4588-4596 (1988). This monoclonal antibody has been reported to target human colon carcinoma xenografts efficiently, and to reduce the growth of such xenografts with good efficacy. Molinolo *et al.*, *Cancer Res.*, 50:1291-1298 (1996); Colcher *et al.*, *J. Natl. Cancer Inst.*,
25 82:1191-1197 (1990). Also, radiolabeled CC49 has been reported to exhibit excellent tumor localization in several ongoing clinical trials.

However, while murine antibodies have applicability as therapeutic agents in humans, they are disadvantageous in some respects. Specifically, murine antibodies, because of the fact that they are of foreign species origin, may be immunogenic in humans. This may result
30 in a neutralizing antibody response (human anti-murine antibody (HAMA) response), which is particularly problematic if the antibodies are desired to be administered repeatedly, *e.g.*, in treatment of a chronic or recurrent disease condition. Also, because they contain murine constant domains they may not exhibit human effector functions.

In an effort to eliminate or reduce such problems, chimeric antibodies have been disclosed. Chimeric antibodies contain portions of two different antibodies, typically of two different species. Generally, such antibodies contain human constant regions and variable regions of another species, typically murine variable regions. For example, some mouse/human chimeric antibodies have been reported which exhibit binding characteristics of the parental mouse antibody, and effector functions associated with the human constant region. *See, e.g.*: U.S. Patent 4,816,567 to Cabilly *et al.*; U.S. Patent 4,978,745 to Shoemaker *et al.*; U.S. Patent 4,975,369 to Beavers *et al.*; and U.S. Patent 4,816,397 to Boss *et al.* Generally, these chimeric antibodies are constructed by preparing a genomic gene library from DNA extracted from pre-existing murine hybridomas. Nishimura *et al.*, *Cancer Res.*, 47:999 (1987). The library is then screened for variable region genes from both heavy and light chains exhibiting the correct antibody fragment rearrangement patterns. Alternatively, cDNA libraries are prepared from RNA extracted from the hybridomas and screened, or the variable regions are obtained by polymerase chain reaction. The cloned variable region genes are then ligated into an expression vector containing cloned cassettes of the appropriate heavy or light chain human constant region gene. The chimeric genes are then expressed in a cell line of choice, usually a murine myeloma line. Such chimeric antibodies have been used in human therapy.

Moreover, the production of chimeric mouse-human antibodies derived from CC49 and CC83, which specifically bind TAG-72, has been reported. In this regard, see *e.g.*, EPO 0,365,997 to Mezes *et al.* (The Dow Chemical Company). One such chimeric CC49 antibody is that produced by the cell line deposited as ATTC No. HB 9884 (Budapest).

Also, Morrison *et al.* report the preparation of several antitumor chimeric monoclonal antibodies. in *Important Advances in Oncology, Recombinant Chimeric Monoclonal Antibodies*, pp. 3-18 (S.A. Rosenberg, ed., 1990) (J.B. Lippincott, Philadelphia, PA). Results of clinical trials with chimeric cMAb-17-1A in patients with metastatic colorectal carcinoma now show that this antibody has a 6-fold longer circulation time and significantly reduced immunogenicity as compared to the murine monoclonal antibody from which it was derived. LoBuglio *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:4220-4224 (1989); Meredith *et al.*, *J. Nucl. Med.*, 32:1162-1168 (1991).

However, while such chimeric monoclonal antibodies typically exhibit lesser immunogenicity, they are still potentially immunogenic in humans because they contain murine variable sequences which may elicit antibody responses. Thus, there is the

possibility that these chimeric antibodies may elicit an anti-idiotypic response if administered to patients. Saleh *et al., Cancer Immunol. Immunother.*, 32:185-190 (1990).

For example, when cB72.3(γ4) was administered to patients with colorectal carcinomas, 62% of such patients elicited a human antimurine antibody (HAMA) response, which included an anti-V-region response. This is disadvantageous because a HAMA response would make repeated antitumor antibody administration potentially ineffective because of an increased antibody clearance from the serum (Saleh *et al., Cancer Immunol. Immunother.*, 32:180-190 (1990)) and also because of potential allergic reactions (LoBuglio *et al., Hybridoma*, 5:5117-5123 (1986).

A number of genetic variants of potential clinical utility have been developed from MAb CC49. These include cCC49, a C_H2 domain-deficient cCC49 (Slavin-Chiorini *et al., Int. J. Cancer*, 53:97-103 (1993)), and a single chain Fv (sFv) (Milenic *et al., Cancer Res.*, 51:6365-6371 (1991); Sawyer *et al., Protein Eng.*, 7:1401-1406 (1994)). These molecules may elicit relatively reduced HAMA responses in patients, since they have shown more rapid plasma and whole body clearance rates in mice and rhesus monkeys, as compared to intact IgG. Slavin-Chiorini *et al.* (1993) (*id.*); Milenic *et al.* (1991) (*id.*). Additionally, novel single-chain immunoglobulin (SCIg) molecules derived from cCC49 have been reported and are encoded by single-gene constructs. One such molecule, SCIgΔC_H1 consists of CC49 sFv linked to the human γ1 Fc region (Shu *et al., Proc. Natl. Acad. Sci. USA*, 90:7915-7999 (1993)) while the other SCIg-IL-2 carries a human interleukin-2 (IL-2) molecule genetically attached to the carboxyl end of the Fc region of SCIgΔC_H1 (Kashmiri *et al., Proc. XVI Intl. Cancer Cong.*, 1:183-187 (1994)). Both SCIgs are comparable to cCC49 in antigen binding and antibody cellular cytolytic activity. The biological activity of the IL-2 is also retained in SCIg-IL-2.

In an effort to alleviate the immunogenicity concerns of chimeric and murine antibodies, the production of "humanized" antibodies is also known. Ideally, "humanization" results in an antibody that is non-immunogenic in humans, with substantially complete retention of the antigen-binding properties of the original molecule. In order to retain all the antigen-binding properties of the original antibody, the structure of its combining-site has to be faithfully reproduced in the "humanized" version. This can potentially be achieved by transplanting the combining site of the nonhuman antibody onto a human framework, either (a) by grafting only the nonhuman CDRs onto human framework and constant regions with or without retention of critical framework residues (Jones *et al., Nature*, 321:522 (1986); Verhoeyen *et al., Science*, 239:1539 (1988)); or (b) by transplanting the entire nonhuman

variable domains (to preserve ligand-binding properties) but also "cloaking" them with a human-like surface through judicious replacement of exposed residues (to reduce antigenicity) (Padlan, *Molec. Immunol.*, 28:489 (1991)).

Essentially, humanization by CDR grafting involves transplanting only the CDRs onto human fragment and constant regions. Theoretically, this should substantially eliminate immunogenicity (except if allotypic or idiotypic differences exist). Jones *et al., Nature,* 321:522-525 (1986); Verhoeyen *et al., Science,* 239:1534-1536 (1988); Riechmann *et al., Nature,* 332:323-327 (1988). While such a technique is effective in some instances, CDR-grafting sometimes does not yield the desired result. Rather, it has been reported that some framework residues of the original antibody may also need to be preserved in order to preserve antigen binding activity. Riechmann *et al.. Nature,* 332:323-327 (1988); Queen *et al., Proc. Natl. Acad. Sci. USA,* 86:10023-10029; Tempest *et al., Biol. Technology,* 9:266-271 (1991); Co *et al., Nature,* 351:501 -502 (1991)).

As discussed, in order to preserve the antigen-binding properties of the original antibody, the structure of its combining site must be faithfully reproduced in the humanized molecule. X-ray crystallographic studies have shown that the antibody combining site is built primarily from CDR residues, although some neighboring framework residues have been found to be involved in antigen binding. Amit *et al.. Science,* 233:747-753 986); Colman *et al., Nature,* 326:358-363 (1987); Sheriff *et al., Proc. Natl. Acad. Sci. USA*, 84:8075-8079 (1987); Padlan *et al., Proc. Natl. Acad Sci. USA.* 86:5938-5942 (1989); Fischmann *et al., J. Biol. Chem..* 266:12915-12920 (1991); Tulip *et al.. J. Molec. Biol.,* 227:122-148 (1992). It has also been found that the structures of the CDR loops are significantly influenced by surrounding framework structures. Chothia *et al., J. Molec. Biol.,* 196:901-917 (1987); Chothia *et al.. Nature,* 342:877-883 (1989); Tramomonteno *et al., J. Molec. BioL.* 215:175-182 (1990).

Small but significant differences in the relative disposition of the variable light chain (V_L) and variable heavy (V_H) domains have been noted (Colman *et al.. Nature.* 326:358-363 (1987)) and those differences are ostensibly due to variations in the residues involved in the interdomain contact (Padlan *et al., Molec. Immunol..* 31:169-217 (1994)).

Furthermore, structural studies of the effect of the mutation of interior residues, in which changes in side chain volume are involved. have shown that the resulting local deformations are accommodated by shifts in side chain positions that are propagated to distant parts of the molecular interior. This suggests that during humanization the interior

residues in the variable domains and in the interface between these domains, or at least the interior volumes, should also be maintained; a humanization protocol in which an interior residue is replaced by one of different physical properties (such as size, charge, or hydrophobicity, etc.), could result in a significant modification of the antigen combining site
5 structure.

One method of identifying the framework residues which need to be preserved is by computer modeling. Alternatively, critical framework residues may potentially be identified by comparing known antibody combining site structures (Padlan, *Molec. Immun.*, 31(3):169-217 (1994)).

10 The residues which potentially affect antigen binding fall into several groups. The first group comprises residues that are contiguous with the combining site surface and which could therefore make direct contact with antigens. They include the amino-terminal residues and those adjacent to the CDRs. The second group includes residues that could alter the structure or relative alignment of the CDRs either by contacting the CDRs or the opposite
15 chains. The third group comprises amino acids with buried side chains that could influence the structural integrity of the variable domains. The residues in these groups are usually found in the same positions (*ibid.*) according to the adopted numbering system. See Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, NIH Pub. No. 91-3242 (5th ed., 1991) (U.S. Dept. Health & Human Services, Bethesda, MD) and Genbank.

20 However, while humanized antibodies are desirable because of their potential low immunogenicity in humans, their production is unpredictable. For example, sequence modification of antibodies may result in substantial or even total loss of antigen binding affinity, or loss of binding specificity. Alternatively, "humanized antibodies" may still exhibit immunogenicity in humans, irrespective of sequence modification.

25 Thus, there still exists a significant need in the art for novel humanized antibodies to desired antigens. More specifically, there exists a need in the art for humanized antibodies specific to TAG-72, because of their potential as immunotherapeutic and immunodiagnostic agents.

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OBJECTS OF THE INVENTION

Toward this end, it is an object of the invention to provide humanized antibodies which are specific to human TAG-72.

More specifically, it is an object of the invention to provide humanized antibodies derived from murine antibodies to TAG-72, and in particular from CC49, a specific murine antibody which binds to TAG-72.

It is also an object of the invention to provide pharmaceutical compositions containing humanized antibodies which are specific to TAG-72. It is a more specific object of the invention to provide pharmaceutical compositions containing humanized antibodies derived from CC49, a murine antibody which specifically binds to TAG-72.

It is another specific object of the invention to provide methods of using humanized antibodies to TAG-72 for treatment of cancers which express TAG-72, in particular human colon cancer.

It is another object of the invention to provide immunodiagnostic compositions for detecting cancer cells which contain a humanized antibody which specifically binds TAG-72, and preferably is derived from CC49, which antibody is in labeled or unlabeled form. It is another object of the invention to provide a method of immunodiagnosis of cancer using compositions which contain a humanized antibody which specifically binds TAG-72, which is in labeled or unlabeled form.

It is still another object of the invention to provide nucleic acid sequences which encode for humanized antibodies to TAG-72 or fragments thereof. It is a more specific object of the invention to provide nucleic acid sequences which encode humanized antibodies derived from CC49, a murine antibody which specifically binds to TAG-72. It is another object of the invention to provide vectors from which may be expressed humanized antibodies to TAG-72, in particular humanized antibodies derived from CC49, a murine antibody which specifically binds to TAG-72.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 aligns amino acid sequences of murine CC49 V_H, the NEWM framework regions encoded by the FR starting material, and the humanized NEWM-based V_H (HuVH) disclosed in Example 1. The CDRs are in the boxes. Murine residues retained in the FRs

are identified with arrow symbols (↑). Murine FR residues retained in alternate versions of the HuVH are identified with letter symbols (A), (S), and (K).

Figure 2 aligns amino acid sequences of murine CC49 V_{κ}, the REI framework regions encoded by the FR starting material, and the humanized REI-based V_{κ} disclosed in Example 1. CDRs are in the boxes.

Figure 3 aligns the variable heavy chain of CC49, the HuCC49 disclosed in Example 1, and NEWM.

Figure 4 aligns the variable light chain of CC49, the HuCC49 disclosed in Example 1, and REI.

Figure 5 contains schematics of the vectors used to express the humanized V_H and V_{κ} shown in Figure 3 and Figure 4.

Figure 6 is an ELISA showing binding of CC49 antibodies HuVHA/MuVK and HuVHA/HuVK to TAG-72.

Figure 7 is an ELISA showing binding of CC49 antibodies MuVH/MuVK and HuVH/HuVK to TAG-72.

Figure 8 is an ELISA showing binding of CC49 antibodies MuVH/MuVK and HuVH/HuVK to TAG-72.

Figure 9 is an ELISA showing binding of CC49 antibodies MuVH/MuVK and HuVHA/HuVK to TAG-72.

Figure 10 is an ELISA showing binding of CC49 antibodies HuVH/HuVK and HuVHK/HuVK to TAG-72.

Figure 11 is an ELISA showing binding of CC49 antibodies HuVHS/HuVK and HuVH/HuVK to TAG-72.

Figure 12 is a Scatchard analysis of humanized (HuVH/HuVK) and chimeric (MuVH/MuVK) CC49 monoclonal antibodies.

Figure 13 presents the single-stranded DNA sequence of the template used to produce the initial humanized NEWM-based VHs, HuVH and HuVHA.

Figure 14 presents the double-stranded DNA sequence of the template used to produce the alternate humanized VHs, HuVHS and HuVHK.

DETAILED DESCRIPTION OF THE INVENTION

Prior to setting forth the invention, definitions of certain terms which are used in this disclosure are set forth below:

Antibody - This refers to single chain, two-chain, and multi-chain proteins and glycoproteins belonging to the classes of polyclonal, monoclonal, chimeric, and hetero immunoglobulins (monoclonal antibodies being preferred); it also includes synthetic and genetically engineered variants of these immunoglobulins. "Antibody fragment" includes Fab, Fab', $F(ab')_2$, and Fv fragments, as well as any portion of an antibody having specificity toward a desired target epitope or epitopes.

Humanized antibody - This will refer to an antibody derived from a non-human antibody, typically murine, that retains or substantially retains the antigen-binding properties of the parent antibody but which is less immunogenic in humans. This may be achieved by various methods including (a) grafting only the non-human CDRs onto human framework and constant regions with or without retention of critical framework residues, or (b) transplanting the entire non-human variable domains, but "cloaking" them with a human-like section by replacement of surface residues. Such methods as are useful in practicing the present invention include those disclosed in Jones *et al.*, Morrison *et al.*, *Proc. NatL Acad. Sci. USA*, 81:6851 -6855 (1984); Morrison and Oi, *Adv. Immunol.*, 44:65-92 (1988); Verhoeyen *et al.*, *Science*, 239:1534-1536 (1988); Padlan, *Molec. Immun.*, 28:489-498 (1991); Padlan, *Molec. Immun.*, 31(3):169-217 (1994).

Complementarity Determining Region, or CDR - The term CDR, as used herein, refers to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site as delineated by Kabat *et al.* (1991).

Framework Region - The term FR, as used herein, refers to amino acid sequences interposed between CDRs. These portions of the antibody serve to hold the CDRs in an appropriate orientation for antigen binding. In the antibodies and antibody fragments of the present invention, the framework regions for the light chain variable region may be selected from the group consisting of human lambda light chain FRs and huma kappa subgroup I, II, and III light chain FRs, whether the amino acid sequences of these FRs remain in their fully human native form or instead contain modifications to their amino acid sequences which are necessary to retain or increase binding affinity and/or binding specificity.

Constant Region - The portion of the antibody molecule which confers effector functions. In the present invention, murine constant regions are substituted with human constant regions. The constant regions of the subject chimeric or humanized antibodies are derived from human immunoglobulins. The heavy chain constant region can be selected from any of the five isotypes: alpha, delta, epsilon, gamma or mu. Further, heavy chains of various subclasses (such as the IgG subclasses of heavy chains) are responsible for different effector functions and thus, by choosing the desired heavy chain constant region chimeric antibodies with desired effector function can be produced.

Preferred constant regions are gamma 1 (IgG1), gamma 3 (IgG3) and gamma 4 (IgG4). More preferred is a constant region of the gamma 1 (IgG1) isotype. The light chain constant region can be of the kappa or lambda type, preferably of the kappa type.

Chimeric antibody - This is an antibody containing sequences derived from two different antibodies, which typically are of different species. Most typically chimeric antibodies comprise human and murine antibody fragments, generally human constant and murine variable regions.

Mammals - Animals that nourish their young with milk secreted by mammary glands, preferably warm blooded mammals, more preferably humans.

Immunogenicity - A measure of the ability of a targeting protein or therapeutic moiety to elicit an immune response (humoral or cellular) when administered to a recipient. The present invention is concerned with the immunogenicity of the subject humanized antibodies or fragments thereof.

Humanized antibody of reduced immunogenicity - This refers to a humanized antibody exhibiting reduced immunogenicity relative to the parent antibody.

Humanized antibody substantially retaining the binding properties of the parent antibody - This refers to a humanized antibody which retains the ability to specifically bind the antigen recognized by the parent antibody used to produce such humanized antibodies. Preferably the humanized antibody will exhibit the same or substantially the same antigen-binding affinity and avidity as the parent antibody, *e.g.*, CC49. Preferably, the affinity of the antibody will at least about 10% of that of the parent antibody. More preferably, the affinity will be at least about 25%, *i.e.* at least two-fold less than the affinity of the parent antibody. Most preferably the affinity will be at least about 50% that of the parent antibody. Methods for assaying antigen-binding affinity are well known in the art and include

half-maximal binding assays, competition assays, and Scatchard analysis. Suitable antigen binding assays are described in this application.

In its broadest embodiment, the present invention is directed to humanized antibodies which specifically bind TAG-72, a pancarcinoma antigen expressed by various human cancers, in particular human colon carcinoma. Preferably, such humanized antibodies will be derived from antibodies having good binding affinity to TAG-72, *e.g.*: B72.3 (Thor *et al.*, *Cancer Res.*, 46:31 18-3127 (1986); Johnson *et al.*, *Cancer Res.*, 46:850-857 (1986)), deposited as ATCC No. HB 8108; CC49 (ATCC No. HB 9459); CC83 (ATCC No. HB 9453); CC46 (ATCC No. HB 9452); CC92 (ATCC No. HB 9454); CC30 (ATCC No. HB 9457); CC11 (ATCC No. 9455); and CC15 (ATCC No. HB 9460); or chimerized forms thereof (see, *e.g.*, EPO 0,365,997 to Mezes *et al.*, The Dow Chemical Company).

Most preferably, such humanized antibodies will be derived from CC49, which has been reported to target human colon carcinoma xenografts efficiently and also to reduce the growth of the xenograft with good efficacy. Molinolo *et al.*, *Cancer Res.*, 50:1291 -1298 (1990); Colcher *et al.*, J. *Natl. Cancer Inst.*, 82:1191 -1197 (1990).

As discussed above, humanized antibodies afford potential advantages over murine and also chimeric antibodies, *e.g.*, reduced immunogenicity in humans. This is advantageous because it should reduce and potentially eliminate the eliciting of a HAMA response when such humanized antibodies are administered *in vivo*, *e.g.*, for treatment of cancer or for diagnosis of cancer, *e.g.*, for tumor imaging.

However, as noted, humanization may in some instances adversely affect antigen binding. Preferably, the humanized antibodies of the present invention which specifically bind TAG-72 will possess a binding affinity for TAG-72 of at least about 10% and more preferably at least about 25% and most preferably at least about 50% that of the TAG-72 antigen binding affinity of the parent murine antibody, *e.g.*, B72.3, CC49, CC46, CC30,CC11, CC15, CC83, or another parent antibody. Most preferably, the humanized antibodies of the present invention will possess a binding affinity for TAG-72 of at least about 10% and more preferably at least about 25% and most preferably at least about 50% that of the TAG-72 antigen binding affinity of either CC49 or a chimeric CC49 antibody.

Preferably, the humanized antibodies of the present invention will bind the same epitope as CC49. Such antibodies can be identified based on their ability to compete with CC49 for binding to TAG-72 or to TAG-72-expressing cancer cells.

In general, the subject humanized antibodies are produced by obtaining nucleic acid sequences encoding the variable heavy and variable light sequences of an antibody which binds TAG-72, preferably CC49, identifying the CDRs in said variable heavy and variable light sequences, and grafting such CDR nucleic acid sequences onto human framework
5 nucleic acid sequences.

Preferably, the selected human framework will be one that is expected to be suitable for *in vivo* administration, *i.e.*, does not exhibit immunogenicity. This can be determined, *e.g.*, by prior experience with *in vivo* usage of such antibodies and by studies of amino acid sequence similarities. In the latter approach, the amino acid sequences of the framework
10 regions of the antibody to be humanized, *e.g.*, CC49, will be compared to those of known human framework regions, and human framework regions used for CDR grafting will be selected which comprise a size and sequence most similar to that of the parent antibody, *e.g.*, a murine antibody which binds TAG-72. Numerous human framework regions have been isolated and their sequences reported in the literature. See, *e.g.*, Kabat *et al.*, (*id.*).This
15 enhances the likelihood that the resultant CDR-grafted "humanized" antibody, which contains the CDRs of the parent (*e.g.*, murine) antibody grafted onto the selected human framework regions will significantly retain the antigen binding structure and thus the binding affinity of the parent antibody. As a result of such studies, the FRs of REI and NEWM antibodies have been identified as having amino acid sequences which are likely to allow the
20 CDRs of CC49 to retain a significant degree of antigen binding affinity. As noted, the selected human framework regions will preferably be those that are expected to be suitable for *in vivo* administration, *i.e.*, not immunogenic. Based on their amino acid sequences, REI and NEWM human framework regions are expected to be substantially non-immunogenic.

Methods for cloning nucleic acid sequences encoding immunoglobulins are well
25 known in the art. Such methods will generally involve the amplification of the immunoglobulin sequences to be cloned using appropriate primers by polymerase chain reaction (PCR). Primers suitable for amplifying immunoglobulin nucleic acid sequences, and specifically murine variable heavy and variable light sequences have been reported in the literature. After such immunoglobulin sequences have been cloned, they will be sequenced
30 by methods well known in the art. This will be effected in order to identify the variable heavy and variable light sequences, and more specifically the portions thereof which constitute the CDRs and FRs. This can be effected by well known methods.

Once the CDRs and FRs of the cloned antibody sequences which are to be humanized have been identified, the amino acid sequences encoding CDRs are then

identified (deduced based on the nucleic acid sequences and the genetic code and by comparison to previous antibody sequences) and the corresponding nucleic acid sequences are grafted onto selected human FRs. This may be accomplished by use of appropriate primers and linkers. Methods for selecting suitable primers and linkers to provide for ligation of desired nucleic acid sequences is well within the purview of the ordinary artisan and include those disclosed in U.S. Patent No. 4,816,397 to Boss *et al.* and U.S. Patent No. 5,225,539 to Winter *et al.*

After the CDRs are grafted onto selected human FRs, the resultant "humanized" variable heavy and variable light sequences will then be expressed to produce a humanized Fv or humanized antibody which binds TAG-72. Typically, the humanized variable heavy and variable light sequences will be expressed as a fusion protein with human constant domain sequences, so that an intact antibody which binds TAG-72 is obtained. However, this is not necessary as the variable heavy and light sequences can also be expressed in the absence of constant sequences to produce a humanized Fv which binds TAG-72. However, fusion of human constant sequences to the humanized variable region(s) is potentially desirable because the resultant humanized antibody which binds TAG-72 will then possess human effector functions such as complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) activity. Such activity has been found in chimeric antibodies, including CC49. The humanized anti-TAG-72 antibodies of the present invention can also support such effector function activity with the added advantage of a greatly decreased risk of a HAMA response.

Methods for synthesizing DNAs encoding a protein of known sequence are well known in the art. Using such methods, DNA sequences which encode the subject humanized V_L and V_H sequences are synthesized. and then expressed in vector systems suitable for expression of recombinant antibodies. This may be effected in any vector system which provides for the subject humanized V_L and V_H sequences to be expressed as a fusion protein with human constant domain sequences and to associate to produce functional (antigen binding) antibodies. Expression vectors and host cells suitable for expression of recombinant antibodies and humanized antibodies in particular, are well known in the art.

The following references are representative of methods and vectors suitable for expression of recombinant immunoglobulins which may be utilized in carrying out the present invention. Weidle *et al.*. *Gene*, 51:21 -29 (1987); Dorai *et al.*, *J. Immunol.*, 13(12):4232-4241 (1987); De Waele *et al.*. *Eur. J. Biochem.*. 176:287-295 (1988); Colcher *et*

al., Cancer Res., 49:1738-1745 (1989); Wood *et al., J. Immunol.*, 145(a):3011 -3016 (1990); Bulens *et al., Eur. J. Biochem.*. 195:235-242 (1991); Beggington *et al., Biol. Technology,* 10:169 (1992); King *et al., Biochem. J.*, 281:317-323 (1992); Page *et al.,Biol. Technology,* 9:64 (1991); King *et al., Biochem. J.*. 290:723-729 (1993); Chaudary *et al., Nature.* 339:394-397 (1989); Jones *et al.. Nature,* 321:522-525 (1986); Morrison and Oi. *Adv. ImmunoL*. 44:65-92 (1988); Benhar *et al., Proc. Natl. Acad. Sci. USA*, 91:12051-12055 (1994); Singer *et al., J. Immunol.*. 150:2844-2857 (1993); Cooto *et al., Hybridoma,* 13(3):215-219 (1994); Queen *et al., Proc. Natl. Acad. Sci. USA*, 86:10029-10033 (1989); Caron *et al., Cancer Res.,* 32:6761 -6767 (1992); Cotoma *et al.*, J. *Immunol. Meth.,* 152:89-109 (1992). Moreover, vectors suitable for expression of recombinant antibodies are commercially available. The vector may, *e.g.*, be a bare nucleic acid segment, a carrier-associated nucleic acid segment, a nucleoprotein, a plasmid, a virus, a viroid, or a transposable element.

Host cells known to be capable of expressing functional immunoglobulins include, *e.g.*: mammalian cells such as Chinese Hamster Ovary (CHO) cells; COS cells; myeloma cells, such as NSO and SP2/0 cells; bacteria such as *Escherichia coli;* yeast cells such as *Saccharomyces cerevisiae;* and other host cells. Of these, CHO cells are used by many researchers given their ability to effectively express and secrete immunoglobulins. NSO cells are one of the preferred types of host cells useful in the present invention.

Essentially, recombinant expression of humanized antibodies is effected by one of two general methods. In the first method, the host cells are transfected with a single vector which provides for the expression of both heavy and light variable sequences optionally fused to selected constant regions. In the second method, host cells are transfected with two vectors, each of which encodes a different variable chain (*i.e.* a variable heavy chain or variable light chain); each variable chain-encoding vector may optionally provide for the expression of the variable chain fused to a selected constant region.

Human constant domain sequences are well known in the art, and have been reported in the literature. Preferred human constant sequences include the kappa and lambda constant light sequences. Preferred human heavy constant sequences include human gamma 1, human gamma 2, human gamma 3, human gamma 4, and mutated versions thereof which provide for altered effect or function, *e.g.*, enhanced *in vivo* half-life or reduced Fc receptor binding.

After expression, the antigen binding affinity of the resulting humanized antibody will be assayed by known methods, *e.g.*, Scatchard analysis. In a particularly preferred embodiment. the antigen-binding affinity of the humanized antibody will be at least 25% of that of the parent antibody, *e.g.*, CC49, *i.e.* a minimum of two-fold less than that of native or chimeric CC49. Most preferably, the affinity of the humanized antibody will be at least about 50% of that of the parent antibody, *e.g.*, CC49.

In some instances, humanized antibodies produced by grafting CDRs (from an antibody which binds TAG-72) onto selected human framework regions may provide humanized antibodies having the desired affinity to TAG-72. However, it may be necessary or desirable to further modify specific residues of the selected human framework in order to enhance antigen binding. This may occur because it is believed that some framework residues are essential to or at least affect antigen binding. Preferably, those framework residues of the parent (*e.g.*, murine) antibody which maintain or affect combining-site structures will be retained. These residues may be identified by X-ray crystallography of the parent antibody or Fab fragment, thereby identifying the three-dimensional structure of the antigen-binding site. Also, framework residues involved in antigen binding may potentially be identified based on previously reported humanized murine antibody sequences. Thus, it may be beneficial to retain such framework residues or others from the parent murine antibody to optimize TAG-72 binding. Preferably, such methodology will confer a "human-like" character to the resultant humanized antibody thus rendering it less immunogenic while retaining the interior and contacting residues which affect antigen-binding.

The present invention further embraces variants and equivalents which are substantially homologous to the humanized antibodies and antibody fragments set forth herein. These may contain, *e.g.*, conservative substitution mutations, *i.e.* the substitution of one or more amino acids by similar amino acids. For example, conservative substitution refers to the substitution of an amino acid with another within the same general class, *e.g.*, one acidic amino acid with another acidic amino acid. one basic amino acid with another basic amino acid. or one neutral amino acid by another neutral amino acid. What is intended by a conservative amino acid substitution is well known in the art.

The phrase "substantially homologous" is used in regard to the similarity of a subject amino acid sequence (of an oligo- or poly-peptide or protein) to a related, reference amino acid sequence. This phrase is defined as at least about 75% Correspondence" -- *i.e.* the state of identical amino acid residues being situated in parallel -- between the subject and

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reference sequences when those sequences are in "alignment," *i.e.* when a minimal number of "null" bases have been inserted in the subject and/or reference sequences so as to maximize the number of existing bases in correspondence between the sequences. "Null" bases are not part of the subject and reference sequences; also, the minimal number of "null" bases inserted in the subject sequence may differ from the minimal number inserted in the reference sequence. In this definition, a reference sequence is considered "related" to a subject sequence where both amino acid sequences make up proteins or portions of proteins which are either αTAG-72 antibodies or antibody fragments with αTAG-72 binding affinity. Each of the proteins comprising these αTAG-72 antibodies or antibody fragments may independently be antibodies or antibody fragments or hi- or multi-functional proteins, *e.g.*, such as fusion proteins, bi- and multi-specific antibodies, single chain antibodies, and the like.

The present invention is further directed to nucleic acid sequences from such humanized antibodies may be expressed, as well as expression vectors which provide for the production of such humanized antibodies in transformed host cells.

In the preferred embodiments, such humanized antibodies and corresponding nucleic acid sequences will be derived from CC49. Most preferably, the humanized heavy chains will have the amino acid sequences set forth in Figure 1 or 3 and the humanized light chains will have the amino acid sequences set forth in Figure 2 or 4. However, as discussed, the invention further contemplates other modifications of these humanized variable heavy and light sequences, *e.g.*, sequences which further comprise one or more conservative amino acid substitutions or sequences which retain one or more additional murine framework residues which affect (enhance) antigen binding, which are alternatives to or supplements for those already shown in these Figures.

The subject humanized antibodies, because they specifically bind TAG-72, a pancarcinoma antigen expressed on many different cancer cell types (*e.g.*, colon carcinoma, breast carcinoma, ovarian carcinoma, prostate carcinoma), and further because they are expected to be significantly non-immunogenic in humans, should be suitable for use as therapeutics for the treatment or prevention of cancers characterized by TAG-72 expression, and as diagnostic agents, *e.g.*, for use in tumor imaging or in the Radioimmunoguided Surgery System (RIGS®). *See* Hinkle *et al, Antibody, Immunoconjugates and Radiopharmaceuticals.* 4(3):339-358 (1991). One skilled in the art would be able, by routine experimentation, to determine what an effective, non-toxic amount of antibody would be for

the purpose of treating cancer. Generally, however, an effective dosage will be in the range of about 0.05 to 100 milligrams per kilogram body weight per day.

The antibodies of the invention may be administered to a mammal in accordance with the aforementioned methods of treatment in an amount sufficient to produce such effect to a therapeutic, prophylactic, or diagnostic effect. Such antibodies of the invention can be administered to such mammal in a conventional dosage form prepared by combining the antibody of the invention with a conventional pharmaceutically acceptable carrier or vehicle, diluent, and/or excipient according to known techniques to form a suspension, injectable solution, or other formulation. It will be recognized by one of skill in the art that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables.

Pharmaceutically acceptable formulations may include, *e.g.*, a suitable solvent, preservatives such as benzyl alcohol if desired, and a buffer. Useful solvent may include, *e.g.*, water, aqueous alcohols, glycols, and phsophonate and carbonate esters. Such aqueous solutions contain no more than 50% by volume of organic solvent. Suspension-type formulations may include a liquid suspending medium as a carrier, *e.g.*, aqueous polyvinylpyrrolidone, inert oils such as vegetable oils or highly refined mineral oils, or aqueous cellulose ethers such as aqueous carboxymethylcellulose. A thickener such as gelatin or an alginate may also be present, one or more natural or synthetic surfactants or antifoam agents may be used, and one or more suspending agents such as sorbitol or another sugar may be employed therein. Such formations may contain one or more adjuvants.

The route of administration of the antibody (or fragment thereof) of the invention may be oral, parenteral, by inhalation or topical. The term parenteral as used herein includes intravenous, intramuscular, subcutaneous, rectal, vaginal or intraperitoneal administration. The subcutaneous, intravenous and intramuscular forms of parenteral administration are generally preferred. The daily parenteral and oral dosage regimens for employing humanized antibodies of the invention prophylactically or therapeutically will generally be in the range of about 0.005 to 100, but preferably about 0.5 to 10, milligrams per kilogram body weight per day.

The antibody of the invention may also be administered by inhalation. By "inhalation" is meant intranasal and oral inhalation administration. Appropriate dosage forms for such

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administration, such as an aerosol formulation or a metered dose inhaler, may be prepared by conventional techniques. The preferred dosage amount of a compound of the invention to be employed is generally within the range of about 0.1 to 1000 milligrams, preferably about 10 to 100 milligrams/kilogram body weight.

5 The antibody of the invention may also be administered topically. By topical administration is meant non-systemic administration. This includes the administration of a humanized antibody (or humanized antibody fragment) formulation of the invention externally to the epidermis or to the buccal cavity, and instillation of such an antibody into the ear, eye, or nose, and wherever it does not significantly enter the bloodstream. By systemic 10 administration is meant oral, intravenous, intraperitoneal, subcutaneous, and intramuscular administration. The amount of an antibody required for therapeutic, prophylactic, or diagnostic effect will, of course, vary with the antibody chosen, the nature and severity of the condition being treated and the animal undergoing treatment, and is ultimately at the discretion of the physician. A suitable topical dose of an antibody of the invention will 15 generally be within the range of about 1 to 100 milligrams per kilogram body weight daily.

Formulations

While it is possible for an antibody or fragment thereof to be administered alone, it is preferable to present it as a pharmaceutical formulation. The active ingredient may comprise, for topical administration, from 0.001% to 10% w/w, e.g., from 1% to 2% by weight 20 of the formulation, although it may comprise as much as 10% w/w but preferably not in excess of 5% w/w and more preferably from 0.1% to 1% w/w of the formulation. The topical formulations of the present invention, comprise an active ingredient together with one or more acceptable carrier(s) therefor and optionally any other therapeutic ingredients(s). The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of 25 the formulation and not deleterious to the recipient thereof.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of where treatment is required, such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear, or nose. Drops according to the present invention may 30 comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active agent. The resulting solution may then be clarified and sterilized by filtration and transferred to the

container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01 %) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

5 Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or
10 an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or
15 non-greasy basis. The basis may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or macrogels. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or
20 non-ionic surface active such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as silicaceous silicas, and other ingredients such as lanolin, may also be included.

Kits according to the present invention include frozen or lyophilized humanized antibodies or humanized antibody fragments to be reconstituted, respectively, by thawing
25 (optionally followed by further dilution) or by suspension in a (preferably buffered) liquid vehicle. The kits may also include buffer and/or excipient solutions (in liquid or frozen form) -- or buffer and/or excipient powder preparations to be reconstituted with water -- for the purpose of mixing with the humanized antibodies or humanized antibody fragments to produce a formulation suitable for administration. Thus, preferably the kits containing the
30 humanized antibodies or humanized antibody fragments are frozen, lyophilized, pre-diluted, or pre-mixed at such a concentration that the addition of a predetermined amount of heat, of water, or of a solution provided in the kit will result in a formulation of sufficient concentration and pH as to be effective for *in vivo* or *in vitro* use in the treatment or diagnosis of cancer. Preferably, such a kit will also comprise instructions for reconstituting and using the

humanized antibody or humanized antibody fragment composition to treat or detect cancer. The kit may also comprise two or more component parts for the reconstituted active composition. For example, a second component part -- in addition to the humanized antibodies or humanized antibody fragments -- may be bifunctional chelant, bifunctional chelate, or a therapeutic agent such as a radionuclide, which when mixed with the humanized antibodies or humanized antibody fragments forms a conjugated system therewith. The above-noted buffers, excipients, and other component parts can be sold separately or together with the kit.

It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of a humanized antibody or humanized antibody fragment of the invention will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular animal being treated, and that such optima can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, *i.e.*, the number of doses of an antibody or fragment thereof of the invention given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

The subject humanized antibodies may also be administered in combination with other anti-cancer agents, *e.g.*, other antibodies or drugs. Also, the subject humanized antibodies or fragments may be directly or indirectly attached to effector having therapeutic activity. Suitable effector moieties include by way of example cytokines (IL-2, TNF, interferons, colony stimulating factors, IL-1, etc.), cytotoxins (Pseudomonas exotoxin, ricin, abrin, etc.), radionuclides, such as ^{90}Y, ^{131}I, ^{99m}Tc, ^{111}In, ^{125}I, among others, drugs (methotrexate, daunorubicin, doxorubicin, etc.), immunomodulators, therapeutic enzymes (*e.g.*, beta-galactosidase), anti-proliferative agents, etc. The attachment of antibodies to desired effectors is well known. See, *e.g.*, U.S. Patent No. 5,435,990 to Cheng *et al.* Moreover, bifunctional linkers for facilitating such attachment are well known and widely available. Also, chelators (chelants and chelates) providing for attachment of radionuclides are well known and available.

Alternatively, the subject humanized antibodies or fragments specific to TAG-72 may be used as immunodiagnostic agents both *in vivo* and *in vitro*. A particularly preferred usage is for *in vivo* imaging of cancer cell lesions which express TAG-72. The subject antibodies are preferred because they should elicit no significant HAMA or allergic response. Thus, they may be used repeatedly to monitor the disease status of a patient.

As noted above, another preferred application of the subject humanized antibodies or fragments thereof is in the Radioimmunoguided System®. This technique, also known as the RIGS® System involves the intravenous administration of a radiolabeled monoclonal antibody or its fragment prior to surgery. After allowing for tumor uptake and blood
5 clearance of radioactivity, the patient is taken to the operating room where surgical exploration is effected with the aid of a hand-held gamma activity probe, *e.g.*, Neoprobe®1000. This helps the surgeon identify the tumor metastases and improve the complications of excision. The RIGS® system is advantageous because it allows for the detection of tumors not otherwise detectable by visual inspection and/or palpation. *See*,
10 O'Dwyer *et al*, *Arch. Surg.*, 121:1 391-1394 (1986). This technique is described in detail in Hinkle *et al*, *Antibody, Immunoconjugates and Radiopharmacouticals*, 4:(3)339-358 (1991) (citing numerous references describing this technique). This reference also discloses the use of this technique with the CC49 monoclonal antibody itself. This technique is particularly useful for cancers of the colon, breast, pancreas, and ovaries.

15 The subject humanized antibodies or humanized antibody fragments thereof radiolabeled with radionuclides which are suitable for *in vivo* administration, *e.g.*, iodine radionuclides such as ^{131}I and ^{125}I; ^{111}In and ^{99m}Tc are also suitable radiolabels.

The subject humanized antibodies may be used alone or in combination with other antibodies. Also, the subject humanized antibodies may be prepared in the form of a
20 diagnostically effective composition. Generally, this will entail the incorporation of diagnostically acceptable carriers and excipients, and labels which provide for detection. Suitable labels include diagnostic radionuclides, enzymes, etc. Methods for using antibodies for tumor imaging are well known in the art.

Without further elaboration, it is believed that one skilled in the art can, using the
25 preceding description, utilize the present invention to its fullest extent. The invention will be further clarified by a consideration of the following examples, which are intended to be purely exemplary of the present invention are thus to be construed as merely illustrative examples and not limitations of the scope of the present invention in any way.

EXAMPLES

Materials and Methods

DNA Template Preparation

All recombination work was performed upon DNA sequences in plasmid M13 vectors.
5 The source of the NEWM framework regions for producing the initial humanized CC49 VH was an M13 construct bearing -- between the M13 *Bam*H I and *Hin*d III sites -- a DNA segment having the nucleotide sequence shown in figure 13. The source of REI framework regions for producing the initial humanized CC49 VL was an M13 construct bearing -- between the M13 *Bam*H I and *Hin*d III sites -- a DNA segment encoding the REI amino acid
10 sequence of Figure 2.

When overlap-extension procedures were used to introduce mutations into a given DNA sequence, double stranded M13 DNA was utilized. In contrast, when extension-ligation procedures were used instead, the oligonucleotides were designed to anneal to only one of the two DNA strands. In this latter procedure, the M13 DNA was first treated to substitute
15 uridine for every thymidine base in the DNA, to produce uridinylated DNA. This was accomplished by transfecting the M13 plasmid DNA into competent cells lacking dUTPase and uracil glycosylase, normally RZ1032 cells (though CJ236 cells available from Bio-Rad of Hercules, CA, are also suitable) by combining the following ingredients.

1 µL of M13 plasmid DNA
20 4 mL of LB broth
40 µL of competent RZ1032 cells.

The culture was shaken for 5 hours at 37°C and the resulting single-stranded plasmid DNA (ssDNA) was isolated and dissolved in 50 µL Tris-EDTA buffer. The DNA was then
25 treated with uracil glycosylase by mixing together:

1 µL uridinylated ssDNA
1 µL 10x glycosylase buffer
1 U uracil glycosylase (Gibco BRL, Gathersburg, MD)
40 µL 25mM MgCl2.

30

This mixture was then incubated at 37°C for one hour and then 6.6 µL 25mM $MgCl_2$ and 9.9µL 1M NaOH. The mixture was then further incubated for 5 minutes at 37°C and 16.5 µL

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of 0.6M HCl was then added to neutralize the mixture. The DNA was then ethanol precipitated and dissolved in water.

M13 Oligonucleotide Primers

5 The following oligonucleotide primers were used throughout the process of preparing the humanized CC49 VHs and VLs exemplified below.

10. 5'-CTAAAACGACGGCCAGT-3';
11. 5'-AACAGCTATGACCATG-3';
385. 5'-GCGGGCCTCTTCGCTATTACGC-3'; and
10 391. 5'-CTCTCTCAGGGCCAGGCGGTGA-3'.

These primers are complementary to regions of the plasmid M13 which are external both to the (NEWM or REI) target framework sequences and to the *Bam*H I site -to- *Hin*d III site section of M13.

15

Murine Variable-Regions

In order to compare the antibody binding characteristics of the antibodies produced according to the examples set forth below, antibodies having a chimeric heavy chain (*i.e.* a heavy chain having a murine CC49 VH region and a human IgG1 constant region) and/or a
20 chimeric light chain (*i.e.* a light chain having a murine CC49 VL and a human κ constant region) were expressed. The source of these chimeric chains was the ATCC-deposited cell line HB9884 (Budapest) which expresses a chimeric CC49 antibody having both chains. The heavy chain of this antibody was termed "MuVH" and the light chain thereof was termed "MuVL."

25

Oligonucleotide Phosphorylation Protocol

Mutating oligonucleotides used in non-overlap extensions were phosphorylated according to the procedure below. In a final volume of 25μL, the following ingredients were combined:

30

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10 pmol of each oligonucleotide,
5μL of a 5x polynucleotide kinase buffer, and
5U of T4 polynucleotide kinase (Gibco BRL).

5

The phosphorylation reaction was started with the addition of the enzyme and allowed to proceed for one hour at 37°C.

Annealing Protocol for Non-Overlap Extension-Ligations

10 The annealing step for non-overlap extension-ligations involved performing one annealing in which all mutation-carrying oligonucleotides and one primer oligonucleotide were annealed to a single stranded DNA template in which all thymidine bases had been replaced with uridine bases. The mutating oligonucleotides were first phosphorylated according to the above oligonucleotide phosphorylation protocol. In a final volume of 20 μL,
15 the following ingredients were combined:

1 pmol of each mutation-carrying phosphorylated oligonucleotide
1 pmol of a primer oligonucleotide
4 μL 5x annealing buffer
0.2 pmol ssU-DNA template.

20

The mixture was then heated to 90°C for 30 sec., then quickly cooled to 70°C, and finally allowed to slowly cool to 37°C.

Extension-Ligation Protocol for Non-Overlap Extension-Ligations

25 After completion of the annealing step in which the primer and phosphorylated mutating oligonucleotides were annealed to the ssU-DNA template, extension-ligation was performed as follows. In a final volume of 30 μL, the following ingredients were combined:

20 μL annealed ssU-DNA (*i.e.* the contents of the above annealing procedure)
2 μL 5x annealing buffer
2 μL 0.1 M dithiothreitol
0.3 μL 0.1M ATP
5 1 μL 6.25mM dNTP mixture of equimolar amounts of dATP, dTTP, dGTP, dCTP
2.5U T7 DNA polymerase (USB, now Amersham Life Sciences, Cleveland, OH)
0.5U T4 DNA ligase (Gibco BRL)
Water to 30μL.

10 This mixture was then incubated at room temperature for 1 hour.

Standard PCR Protocols

The following procedure was used, alternately, both to amplify the non-overlap extension-ligation DNA sequences and to perform extension of each overlap DNA 15 sequence. In a final volume of 50μL, the following ingredients were combined:

2 μL template DNA (either annealed ssU-DNA or non-annealed ssDNA)
5 μL 10x Vent buffer (NEB, *i.e.* New England Biolabs, Beverly, MA) or
10x Thermalase buffer (IBI of New Haven, CT)
2 μL 6.25mM dNTP mixture of equimolar amounts of dATP, dTTP, dGTP, dCTP
20 25 pmol of one oligonucleotide primer
25 pmol of either a mutation-carrying oligonucleotide (for overlap-extension) or
a second oligonucleotide primer
1 U Vent DNA polymerase (NEB) or Thermalase DNA polymerase (IBI).

25 Reactions were initiated with the addition of the DNA polymerase and then treated with about 15 cycles of: (1) 94°C for 30 sec., (2) 50°C for 30 sec., and (3) 30-60 seconds at either 75°C (for Vent DNA polymerase) or 72°C (for Thermalase). Reactions were brought to completion with 5 minutes at a constant temperature of either 75°C For Vent DNA polymerase) or 72°C (for Thermalase).

30

PCR Overlap-Extension Amplification Protocol

After a pair of PCR reactions were performed -- one for each of the two (partially complementary) overlapping DNA segments -- the two resulting segments were joined according to the following PCR procedure. In a final volume of 50µL, the following ingredients were mixed:

1 µL of each overlap DNA (from the above overlap PCR extension reactions)
5 µL 10x Vent buffer (NEB) or Thermalase buffer (IBI)
2 µL 6.25mM dNTP mixture of equimolar amounts of dATP, dTTP, dGTP, dCTP
25 pmol of each oligonucleotide primer used in the overlap PCR extensions
1 U Vent DNA polymerase (NEB) or Thermalase DNA polymerase (IBI).

Reactions were initiated with the addition of the DNA polymerase and then treated with about 15 cycles of: (1) 94°C for 30 sec., (2) 50°C for 30 sec., and (3) 30-60 seconds at either 75°C (for Vent DNA polymerase) or 72°C (for Thermalase). Reactions were brought to completion with 5 minutes at a constant temperature of either 75°C (for Vent DNA polymerase) or 72°C (for Thermalase).

Transfer of Humanized CC49 Variable Region DNA Sequences from M13 to pSV Vectors and Subsequent Antibody Expression

Humanized antibodies were expressed in pSV vectors grown in NSO cells. The humanized variable region constructs which were produced in the plasmid, M13, were digested with 10U each of *Hind* III and *Bam*H I (both from BRL, *i.e.* Gibco BRL) for 1 hour at 37°C in a final volume of 100 µL with Tris-EDTA buffer. The resulting DNA fragments were then run on a low melting point agarose gel, the band containing the humanized construct DNA was cut out, and the DNA was purified using an ELUTIP 'd' column with 20 µL Tris-EDTA buffer. 10 µL of the purified DNA preparation was then combined with 1 µL of a *Hind* III and *Bam*H I-digested pSV preparation, 3 µL of 5x ligase buffer, and 1U of T4 DNA ligase (BRL), in order to insert the construct into a pSV plasmid. Humanized CC49 VH constructs were inserted into pSVgpt vectors bearing a human IgG1 heavy chain constant region; the pSVgpt vector used is the "aLYS-30" shown in Figure 5. Humanized CC49 VL constructs were inserted into pSVhyg vectors bearing a human κ light chain constant domain; the pSVhyg vector used in the "aLys-17" shown in Figure 5. Each humanized

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variable region construct was inserted adjacent to the respective constant region, *i.e.* so as to replace either the HuVHLYS or the HuVLLys segment illustrated in Figure 5.

The resulting vectors were transfected into NSO cells as follows. About 3 μg of the VH vector, or about 6μg of the VL vector, produced by the pSV-insertion procedures, was
5 then linearized by digestion with 10 U Pvu I (Gibco BRL). The digested DNA was then precipitated with ethanol and redissolved in 50 μL of water. NSO cells were collected by centrifugation and resuspended in 0.5mL Dulbecco's Modified Eagle's Medium (DMEM) and then transferred to a Gene Pulser cuvette (Bio-Rad). The DNA from both one VH and one VL construct was gently mixed with the cells by pipetting and the cuvette was left on ice for 5
10 minutes. Next, the cuvette was inserted between the electrodes of the Bio-Rad Gene Pulser and a single pulse of 170V at 960 μF was applied. The contents of the cuvette were then transferred to a flask containing 20mL DMEM and the cells were allowed to rest for 1-2 days at 37°C. Cells were again harvested by centrifugation and resuspended in 36mL selective DMEM. 1.5mL aliquots of this resuspension were placed in each well of a 24-well plate and
15 incubated at 37°C for 4 days, at which time the medium in each well was replaced with 1.5mL of fresh selective DMEM. After 6 more days of incubation at 37°C, surviving cell colonies were visible to the naked eye and the supernatants of each well were assayed for antibody production. Both whole antibody production (*i.e.* without purification) and purified antibody production were assayed. To obtain purified antibodies, the supernatants were
20 passed through a protein A column.

<u>ELISA Assay Protocols</u>

Antibody concentrations and antibody binding characteristics were tested using enzyme-linked immunosorbent assay (ELISA) procedures which are set forth as follows.

<u>Measurement of IgG concentration</u>

25 The concentration of IgG secreted from transfected cells was measured using an enzyme-linked immunosorbent assay (ELISA) procedure which is set forth as follows.

Polyvinyl chloride (PVC) microtiter plates (Dynatech Laboratories, Chantilly, VA, catalog # 001-010-2101) were coated with goat anti-human IgG (10mg/mL, GAHIG, Southern Biotechnology Associates, Inc., Birmingham, AL, catalog # 2010-01) diluted with
30 Milli-Q® water and placed on the plates using 50mL/well. Plates were air-dried overnight at ambient temperature or at 37°C for 3 hours. Prior to use, non-specific binding was blocked the addition of 0.2 mL/well of 1% (w/v) bovine serum albumin (Sigma, St. Louis, MO catalog # A7888) in phosphate buffered saline (Sigma, catalog # 1000-3) (PBS/BSA). All

incubations were carried out in a humidified container. Plates were incubated for 1-2 hours at 37°C and the blocking solution removed prior to sample addition. Two-fold serial dilutions of samples or a standard IgG solution set at 500 ng/mL (50 μL/well) were made in triplicate in the PBS/BSA solution. The plate was incubated at 37°C for 3 hours or overnight at 4°C.
5 The plate was washed 3 times with 0.025% Tween-20 (v/v, Sigma) using an automatic plate washer. 50 μL/well of 1:1000 dilution of a goat anti-human IgG conjugated to Horseradish Peroxidase (Southern Biotechnology Associates Inc.) was added and incubated at 37°C for 1.5 hours. The wells were washed 3 times with 0.025% Tween-20 (v/v, Sigma) using an automatic plate washer and 50 μL/well OPD substrate buffer added. The color was
10 developed for 4 minutes, stopped with 12.5 μL 12.5% H_2SO_4 and the absorbance at 492 nm read. The concentration of IgG in the test sample was estimated by comparison of the mean of the optical densities to a standard curve constructed from the standard IgG.

Determination of relative affinities of humanized antibodies

15 Antibody binding characteristics were tested in an ELISA using partially purified TAG-72 antigen immobilized on Polyvinyl chloride (PVC) microtiter plates (Dynatech Laboratories, Chantilly, VA, catalog # 001-010-2101)

PVC plates were coated with 50 μL/well TAG-72 (Dow Chemical, lot #040191), diluted 1:300 in Milli-Q water. Plates were air-dried overnight at ambient temperature or at
20 37°C for 3 hours. Prior to use, non-specific binding was blocked the addition of 0.2 mL/well of 1% (w/v) bovine serum albumin (Sigma, St. Louis, MO catalog # A7888) in phosphate buffered saline (Sigma, catalog # 1000-3) (PBS/BSA). Plates were incubated for 1-2 hours at 37°C and the blocking solution removed prior to sample addition. All incubations were carried out in a humidified container. Two-fold serial dilutions (starting concentration range
25 of 1.0μg/ml - 10μg/ml) of the samples to be tested in the PBS/BSA solution were added to triplicate wells of the TAG-coated plate (50 μL/well). The plate was incubated overnight at 4°C or 1-2 hours at 37°C. The plate was washed 3-times with 0.025% Tween-20 (v/v, Sigma) using an automatic plate washer. 50 μL/well of 1:1000 dilution of a goat anti-human IgG conjugated to Horseradish Peroxidase (Southern Biotechnology Associates Inc.) was
30 added and incubated at 37°C for 1.5 hours. The wells were washed 3 times with 0.025% Tween-20 (v/v, Sigma) using an automatic plate washer and 50 μL/well OPD substrate buffer added. The color was developed for 4 minutes, stopped with 12.5 μL 12.5% H_2SO_4 and the absorbance at 492 nm read.

Determination of Affinity Constants for binding to TAG-72

Two-fold dilutions of purified Hu-CC49 were prepared in PBS/BSA over a range of 1.0μg/ml - 0.003μg/ml and samples (20 μL/well) were applied in triplicate to TAG coated PVC prepared and blocked as described *supra*. Plates were incubated overnight at 4°C. Following this incubation, samples were transferred from the plate to the corresponding wells on the GAHIG-coated trap plate. The original TAG plate was washed 3-times with 0.025% Tween-20 (v/v, Sigma, catalog # P1379) using an automatic plate washer. An ^{125}I-labeled goat anti-human IgG probe (ICN Biomedicals, Inc., catalog # 68088) was diluted to 75,000 cpm/25μL in PBS/BSA and added (25μL/well) to all wells. This TAG plate was incubated for 1 hour at 37°C.

After a 1 hour incubation at 37°C, the trap plate was washed as described above and ^{125}I-labeled GAHIG probe was added. This plate was incubated for 1 hour at 37°C. Both plates (TAG and GAHIG-trap) containing probe were then washed with a microplate washer to remove the unbound probe. A plate cutter (D. Lee, Sunnyvale, CA, Model HWC-4) was used to separate the wells from the plate frame. The radioactivity in each well was quantified by a gamma counter. The resulting data was analyzed according to the method of Scatchard *(Ann. NYAcad.,* 51:600-672 (1946)).

Example 1

Preparation of CDR-grafted (initial humanized) antibody from murine CC49

We describe in this Example the construction of humanized CC49 Mabs (CC49 HuVH/HuVK) using the V_L and V_H frameworks of human Mabs REI and NEWM, respectively. The CDRs for murine CC49 were grafted onto human frameworks according to known methods as discussed *supra*. In particular, human frameworks were selected from antibodies which, based on previous studies, were predicted to be suitable, *i.e.* which should not adversely affect antigen binding and not exhibit significant immunogenicity in humans. The human frameworks selected for the variable heavy and variable light chains, respectively, were NEWM and REI. In the production of the initial version of the humanized VH, certain murine framework residues were also retained which, based on previous studies, might allow retention of antigen binding properties. Specifically, residues Y27, T30, A72, F95, and T97 of the murine heavy chain were initially retained. Concurrently, an alternate version of the humanized VH was produced which retained, in addition, the murine framework residue A24.

The production of these NEWM-grafted humanized CC49 VHS was accomplished according to the annealing and extension-ligation protocols described above, using a single-stranded M13 DNA template bearing, between the *Hin*d III and *BamH* I sites thereof, a DNA segment having the nucleotide sequence shown in Figure 13. In this procedure, Primer 11 was used in conjunction with a set of mutating oligonucleotides. These mutating oligonucleotides were designed and synthesized with the following sequences:

1a. 5'-GCTGTCTCACCCAGTGAATTGCATGGTC**AGT**GAAGGT**GTA**GCCAGA CACGGTGCAGGTCA-3';

1b. 5'-GCTGTCTCACCCAGTGAATTGCATGGTC**AGT**GAAGGT**GTA**GCCAGA **CGC**GGTGCAGGTCA-3';

2. 5'-CTGGTGTC**TGC**CAGCATTGTCACTCTCCCCTTGAACCTCTCATTGTATTT AAAATCATCATTTCCGGGAGAAAAATATCCAATCCACTCAAGAC-3'; and

3. 5'-GGACCCTTGGCCCCAGTAGGCCATATTCAGGGATCT**TGT**ACA**GAA**ATA GACCGCGGTGTC-3'

Codons which were designed into the oligonucleotides in order to retain murine FR amino acids are shown in bold-face type. After extension-ligation, amplifying PCR was performed using the standard PCR protocol with Vent DNA polymerase. The use of two versions of mutating oligonucleotide 1 resulted in the formation of two initial humanized V_Hs. These were named "CC49 NMVH," also called "HuVH," (for constructs incorporating oligonucleotide 1a) and "HuVHA" (for constructs incorporating oligonucleotide 1b).

In the production of the initial version of the humanized VL, no uniquely murine framework residues were retained. The production of the REI-grafted humanized CC49 V, was accomplished according to the annealing and extension-ligation protocols described above, using a ssM13 template bearing, between the *Hin*d III and *Bam*H I sites thereof, a ssU-DNA segment encoding the REIVK sequence shown in Figure 2. In this procedure, Primer 385 was used in conjunction with a set of mutating oligonucleotides. These mutating oligonucleotides were designed and synthesized with the following sequences:

21. 5'-GTTCTTCTGATTACCACTGTATAAAAGACTTTGACTGGAC-3';

22. 5'-CAGATTCCCTAGCGGATGCCCAGTAG-3';

23. 5'-TTCTACTCACGTGTGATTTGCAGCTTGGTCCCTTGGCCGAACGTGAG GGGATAGGAATAGTATTGCTGGCAGTAGTAG-3'; and

24. 5'-GCTCTGGGTCATCTGGATGTCGG-3'.

After extension-ligation, amplifying PCR was performed using the Thermalase standard PCR protocol described above. The resulting humanized V. was named "CC49 REVK" and was also called "HuVK."

The two heavy chain constructs, HuVH and HuVHA, and the light chain construct, HuVH, which were situated in M13 vectors, were grown and expressed in TG1 cells. The polypeptide expression products of these constructs were sequenced and the amino acid sequences of these constructs are presented in Figures 1 and 2.

These DNA constructs were then inserted into pSV expression vectors as described above. Combinations of these with each other or with the ATCC (Budapest) HB 9884 DNA sequences encoding the chimeric MuVH or MuVL were then inserted into NSO cells. Specifically, the following four combinations of heavy and light chain constructs were separately transfected into NSO cells as described above: HuVHA and MuVK, HuVHA and HuVK, MuVH and MuVK, and HuVH and HuVK. These combinations were expressed and the resulting antibodies were then tested for antigen binding characteristics using the ELISA assay set forth above. The results of these assays are shown in Figures 6-9.

The data in Figure 6 show that, with HuVHA, the HuVK humanized light chain functions as well as the MuVK chimeric light chain. Figures 7 and 8 indicate that the fully humanized HuVH/HuVK antibody binds TAG-72 approximately 2-fold less than the chimeric MuVH/MuVK antibody. Also, Figure 9 suggests that the A24 mutation produces no enhancement in antigen binding; rather, the A24 mutation causes an approximate 2-fold reduction in affinity as measured by this ELISA assay.

Further Development of Humanized CC49

Because it appears that the HuVK humanized light chain functions as well as the MuVK chimeric light chain, further work was directed to making alternate mutated versions of the HuVH humanized heavy chain. A first variant of HuVH was made by replacing the lysine residue shown at position 76 of the CC49 NMVH in Figure 1 with the murine FR residue, serine. This was achieved by the Vent DNA polymerase PCR overlap extension protocol described above, using two primer-and-mutating oligonucleotide pairs -- oligonucleotides 10 and 4, and oligonucleotides 11 and 5. Each pair was used in conjunction with one of the strands of a dsDNA template -- situated between the *Hin*d III and *Bam*H I sites of plasmid M13 -- and having the nucleotide sequence shown in Figure 14 (the upper strand was used with pair 11 & 5). Mutating oligonucleotides 4 and 5 were designed and synthesized with the following sequences:

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4. 5'-AGACACCAGC**AGC**AACCAGTTCAG-3'; and

5. 5'-GCTGAACTGGTT**GCT**GCTGGTGTC-3'.

The serine residue codons are shown in bold-face type. The resulting humanized CC49 V_H was named "HuVHS."

5 A second variant of HUVH was made by replacing the threonine residue shown at position 74 of the CC49 NMVH in Figure 1 with the murine FR residue, lysine. This was achieved by the Vent DNA polymerase PCR overlap extension protocol described above, using two primer-and-mutating oligonucleotide pairs -- oligonucleotides 10 and 6, and oligonucleotides 11 and 7. Each pair was used in conjunction with one of the strands of a 10 dsDNA template -- situated between the *Hin*d III and *Bam*H I sites of plasmid M13 -- and having the nucleotide sequence shown in Figure 14 (the upper strand was used with pair 11 & 7). Mutating oligonucleotides 6 and 7 were designed and synthesized with the following sequences.

6. 5'-CTGGCAGAC**AAG**AGCAAGAACCAG-3'.

15 7. 5'-TGGTTCTTGCT**CTT**GTCTGCCAGC-3'.

The lysine residue codons are shown in bold-face type. The resulting humanized CC49 VH was named "HuVHK."

The two heavy chain constructs, HUVHS and HUVHK, which were situated in M13 vectors, were grown and expressed in TG1 cells. The polypeptide expression products of 20 these constructs were sequenced and the amino acid sequences of these constructs are presented in Figure 1.

These DNA constructs were then inserted into pSV expression vectors as described above. Combinations of these with HUVK were then inserted into NSO cells. These combinations were expressed and the resulting antibodies were then tested for antigen 25 binding characteristics using the ELISA assay set forth above. The results of these assays are shown in Figures 10 and 11. These figures show that neither the K74 nor S76 mutation resulted in enhanced antigen binding; in fact the S76 mutation caused an approximate 2-fold reduction in affinity as measured by this ELISA assay.

30

Example 2

Measurement of affinity constant for the humanized CC49 antibody obtained in Example 1

The affinity constant of the final humanized CC49 antibody, CC49 HuVH/HuVK, of Example 1 (having variable heavy and variable light chain sequences shown in Figures 3 and 4, respectively) were measured according to the ELISA assay protocol set forth above. The ATCC (Budapest) HB 9884 chimeric CC49 antibody was used as an internal control. This ELISA assay was performed repeatedly using purified samples of humanized and chimeric CC49 monoclonal antibodies in order to substantiate the accuracy of the values obtained and to account for inherent assay-to-assay variability. Typical results from such an analysis are shown in Figure 12. A summary of the results obtained during these analyses is shown in Table 1.

TABLE 1

Summary of Affinity Constant Analysis of Humanized & Chimeric CC49

	Chimeric CC49[1]	CC49 HuVH/HuVK
Affinity Constant (Ka)	$7.62 \times 10^{9} M^{-1}$	$4.27 \times 10^{9} M^{-1}$
±standard deviation	$3.94 \times 10^{9} M^{-1}$	$2.57 \times 10^{9} M^{-1}$
number of analyses	7	8

These results demonstrate that the humanized anti-TAG-72 antibody HuVH/HuVK (having the variable heavy and variable light sequences shown in Figures 3 and 4, respectively) has a binding affinity approximating that of a chimeric CC49 antibody (MuVh/MuVK). Therefore, it is expected that this humanized antibody will effectively target TAG-72-expressing carcinomas *in vivo*. Also, based on its sequence this antibody should exhibit little or no immunogenicity in humans, and exhibit advantageous plasma clearance, metabolic properties, and effective tumor targeting in relation to the murine CC49, and also in relation to chimeric versions thereof.

A murine plasmacytoma cell line which produces this humanized CC49 antibody was deposited with the American Type Culture Collection (12301 Parklawn Drive, Rockville, Maryland 20852, on October 16, 1996) and this cell line was accorded accession number

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ATCC CRL-12209. This deposit was made in accordance with the Budapest Treaty. This deposited cell line will be made irrevocably available, without restriction, upon issuance of a patent to this application or any other application claiming priority to this application under 35 U.S.C. § 120.

5 Based on the foregoing, it will be appreciated that the humanized antibodies disclosed in Examples 1-3, exhibit antigen-binding characteristics, *i.e.* TAG-72 affinities comparable to the parent monoclonal antibody, nCC49 (murine antibody), and to chimeric antibodies derived from nCC49, *e.g.*, cCC49. Moreover, based on the foregoing results, these antibodies possess properties which will render them well suited for usage as *in vivo* 10 diagnostics or therapeutics, *e.g.*, improved serum clearance, metabolic properties, and little or no immunogenicity in humans.

These properties are highly significant since because these properties will enable the subject humanized antibodies to be administered repeatedly, in large dosages, and over a prolonged period of time without significant adverse effects, *e.g.*, a HAMA response or 15 non-specific cytotoxicity. This is important for cancer treatment as well as for cancer diagnosis as it enables these antibodies to be used over prolonged time periods. Moreover, the clearance properties of the subject human antibodies will enable these antibodies to effectively target desired target sites, *e.g.*, TAG-72 expressing carcinomas (because of the effects of serum clearance on targeting efficiency). Therefore, the humanized antibodies of 20 the present invention comprise a substantial improvement in relation to previously disclosed antibodies specific to TAG-72.

Other embodiments of the invention will be apparent to those skilled in the art from a consideration of this specification or practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope 25 and spirit of the invention being indicated by the following claims.

SEQUENCE LISTING

GENERAL INFORMATION:
APPLICANT:
NAME: The Dow Chemical Company
STREET: 1790 Bldg. Washington Street
CITY: Midland
STATE: MI
COUNTRY: U.S.A.
POSTAL CODE: 48674
TELEPHONE: 517-636-1687
TELEFAX: 517-638-9786

TITLE OF INVENTION: High Affinity Humanized Anti-TAG-72 Monoclonal Antibodies

NUMBER OF SEQUENCES: 33

COMPUTER READABLE FORM:
MEDIUM TYPE: 3-1/2" diskette
COMPUTER: IBM PC Compatible
OPERATING SYSTEM: MS-DOS
SOFTWARE: PatentIn

INFORMATION FOR SEQ ID NO:1:

SEQUENCE CHARACTERISTICS:
LENGTH: 115
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Murine CC49 VH
LOCATION: 1..115

DESCRIPTION OF SEQ ID NO: 1:

```
Gln Val Gln Leu Gln Gln Ser Asp Ala Glu Leu Val Lys Pro Gly Ala
                5                   10                  15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp His
            20                  25                  30

Ala Ile His Trp Val Lys Gln Asn Pro Glu Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Phe Ser Pro Gly Asn Asp Asp Phe Lys Tyr Asn Glu Arg Phe
    50                  55                  60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Val Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
                85                  90                  95
```


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```
Thr Arg Ser Leu Asn Met Ala Tyr Trp Gly Gln Gly Thr Ser Val Thr
            100                 105                 110

Val Ser Ser
        115
```

INFORMATION FOR SEQ ID NO:2:

SEQUENCE CHARACTERISTICS:
LENGTH: 115
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: NEWM VH FR template
LOCATION: 1..115

DESCRIPTION OF SEQ ID NO: 2:

```
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
                5                   10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Xaa Xaa Xaa Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
        35                  40                  45

Gly Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
    50                  55                  60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Thr Ser Lys Asn Gln Phe Ser
65                  70                  75                      80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Xaa Xaa
                85                  90                  95

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Trp Gly Gln Gly Ser Leu Val Thr
            100                 105                 110

Val Ser Ser
        115
```

INFORMATION FOR SEQ ID NO:3:

SEQUENCE CHARACTERISTICS:
LENGTH: 115
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized CC49 VH, HuVH
LOCATION: 1..115

DESCRIPTION OF SEQ ID NO: 3:

```
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
                 5                  10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Thr Phe Thr Asp His
             20                  25                  30

Ala Ile His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
         35                  40                  45

Gly Tyr Phe Ser Pro Gly Asn Asp Asp Phe Lys Tyr Asn Glu Arg Phe
     50                  55                  60

Lys Gly Arg Val Thr Met Leu Ala Asp Thr Ser Lys Asn Gln Phe Ser
 65                  70                  75                  80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Phe Cys
                 85                  90                  95

Thr Arg Ser Leu Asn Met Ala Tyr Trp Gly Gln Gly Ser Leu Val Thr
            100                 105                 110

Val Ser Ser
        115
```

INFORMATION FOR SEQ ID NO:4:

SEQUENCE CHARACTERISTICS:
LENGTH: 115
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized CC49 VH, HuVHA
LOCATION: 1..115

DESCRIPTION OF SEQ ID NO: 4:

```
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
                 5                  10                  15

Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Tyr Thr Phe Thr Asp His
             20                  25                  30

Ala Ile His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
         35                  40                  45

Gly Tyr Phe Ser Pro Gly Asn Asp Asp Phe Lys Tyr Asn Glu Arg Phe
     50                  55                  60

Lys Gly Arg Val Thr Met Leu Ala Asp Thr Ser Lys Asn Gln Phe Ser
 65                  70                  75                  80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Phe Cys
                 85                  90                  95
```

```
Thr Arg Ser Leu Asn Met Ala Tyr Trp Gly Gln Gly Ser Leu Val Thr
            100                 105                 110

Val Ser Ser
        115
```

INFORMATION FOR SEQ ID NO:5:

SEQUENCE CHARACTERISTICS:
LENGTH: 115
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized CC49 VH, HuVHS
LOCATION: 1..115

DESCRIPTION OF SEQ ID NO: 5:

```
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
                  5                  10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Thr Phe Thr Asp His
             20                  25                  30

Ala Ile His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
         35                  40                  45

Gly Tyr Phe Ser Pro Gly Asn Asp Asp Phe Lys Tyr Asn Glu Arg Phe
     50                  55                  60

Lys Gly Arg Val Thr Met Leu Ala Asp Thr Ser Ser Asn Gln Phe Ser
 65                  70                  75                  80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Phe Cys
                 85                  90                  95

Thr Arg Ser Leu Asn Met Ala Tyr Trp Gly Gln Gly Ser Leu Val Thr
            100                 105                 110

Val Ser Ser
        115
```

INFORMATION FOR SEQ ID NO:6:

SEQUENCE CHARACTERISTICS:
LENGTH: 115
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized CC49 VH, HuVHK
LOCATION: 1..115

DESCRIPTION OF SEQ ID NO: 6:

```
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
                 5                  10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Thr Phe Thr Asp His
            20                  25                  30

Ala Ile His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Phe Ser Pro Gly Asn Asp Asp Phe Lys Tyr Asn Glu Arg Phe
    50                  55                  60

Lys Gly Arg Val Thr Met Leu Ala Asp Lys Ser Lys Asn Gln Phe Ser
65                  70                  75                  80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Phe Cys
                85                  90                  95

Thr Arg Ser Leu Asn Met Ala Tyr Trp Gly Gln Gly Ser Leu Val Thr
            100                 105                 110

Val Ser Ser
        115
```

INFORMATION FOR SEQ ID NO:7:

SEQUENCE CHARACTERISTICS:
LENGTH: 113
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Murine CC49 VK
LOCATION: 1..113

DESCRIPTION OF SEQ ID NO: 7:

```
Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Pro Val Ser Val Gly
                 5                  10                  15

Glu Lys Val Thr Leu Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
            20                  25                  30

Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
        35                  40                  45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Ala Arg Glu Ser Gly Val
    50                  55                  60
```

```
Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser
 65                 70                  75                  80

Ile Ser Ser Val Lys Thr Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
                85                  90                  95

Tyr Tyr Ser Tyr Pro Leu Thr Phe Gly Ala Gly THr Lys Leu Val Leu
            100                 105                 110

Lys
```

INFORMATION FOR SEQ ID NO:8:

SEQUENCE CHARACTERISTICS:
LENGTH: 113
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: REI VK FR template
LOCATION: 1..113

DESCRIPTION OF SEQ ID NO: 8:

```
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
                5                   10                  15

Asp Arg Val Thr Ile Thr Cys Lys Ser Ser Xaa Xaa Xaa Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Thr Pro Gly Lys
        35                  40                  45

Ala Pro Lys Leu Leu Ile Tyr Trp Ala Xaa Xaa Xaa Glu Ser Gly Val
    50                  55                  60

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe THr
 65                 70                  75                  80

Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Xaa Xaa
                85                  90                  95

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Phe Gly Gln Gly Thr Lys Leu Gln Ile
            100                 105                 110

Thr
```

INFORMATION FOR SEQ ID NO:9:

SEQUENCE CHARACTERISTICS:
LENGTH: 113
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Humanized CC49 VK, HuVK
LOCATION: 1..113

DESCRIPTION OF SEQ ID NO: 9:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
                 5                  10                  15

Asp Arg Val Thr Ile Thr Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
            20                  25                  30

Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Thr Pro Gly Lys
        35                  40                  45

Ala Pro Lys Leu Leu Ile Tyr Trp Ala Ser Ala Arg Glu Ser Gly Val
    50                  55                  60

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr
65                  70                  75                  80

Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln
                85                  90                  95

Tyr Tyr Ser Tyr Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile
            100                 105                 110

Thr
```

INFORMATION FOR SEQ ID NO:10:

SEQUENCE CHARACTERISTICS:
LENGTH: 115
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Murine CC49 VH
LOCATION: 1..115

DESCRIPTION OF SEQ ID NO:10:

```
Gln Val Gln Leu Gln Gln Ser Asp Ala Glu Leu Val Lys Pro Gly Ala
                 5                  10                  15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp His
            20                  25                  30

Ala Ile His Trp Val Lys Gln Asn Pro Glu gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Phe Ser Pro Gly Asn Asp Asp Phe Lys Tyr Asn Glu Arg Phe
    50                  55                  60
```

```
Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
 65                  70                  75                  80

Val Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
                 85                  90                  95

Thr Arg Ser Leu Asn Met Ala Tyr Trp Gly Gln Gly Thr Ser Val Thr
            100                 105                 110

Val Ser Ser
        115
```

INFORMATION FOR SEQ ID NO:11:

SEQUENCE CHARACTERISTICS:
LENGTH: 115
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: HuCC49 VH
LOCATION: 1..115

DESCRIPTION OF SEQ ID NO:11:

```
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
                  5                  10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Thr Phe Thr Asp His
             20                  25                  30

Ala Ile His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
         35                  40                  45

Gly Tyr Phe Ser Pro Gly Asn Asp Asp Phe Lys Tyr Asn Glu Arg Phe
     50                  55                  60

Lys Gly Arg Val Thr Met Leu Ala Asp Thr Ser Lys Asn Gln Phe Ser
 65                  70                  75                  80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Phe Cys
                 85                  90                  95

Thr Arg Ser Leu Asn Met Ala Tyr Trp Gly Gln Gly Ser Leu Val Thr
            100                 105                 110

Val Ser Ser
        115
```

INFORMATION FOR SEQ ID NO:12:

SEQUENCE CHARACTERISTICS:
LENGTH: 117
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: NEWM VH
LOCATION: 1..117

DESCRIPTION OF SEQ ID NO:12:

```
Gln Val Gln Leu Glu Gln Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
                5                   10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Ser Thr Phe Ser Asn Asp
            20                  25                  30

Tyr Tyr Thr Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Val Phe Tyr His Gly Thr Ser Asp Asp Thr Thr Pro Leu Arg
    50                  55                  60

Ser Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65                  70                  75                  80

Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95

Arg Asn Leu Ile Ala Gly Cys Ile Asp Val Trp Gly Gln Gly Ser Leu
            100                 105                 110

Val Thr Val Ser Ser
        115
```

INFORMATION FOR SEQ ID NO:13:

SEQUENCE CHARACTERISTICS:
LENGTH: 113
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Murine CC49 VL
LOCATION: 1..113

DESCRIPTION OF SEQ ID NO:13:

```
Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Pro Val Ser Val Gly
                5                   10                  15

Glu Lys Val Thr Leu Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
            20                  25                  30
```

```
Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
        35                  40                  45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Ala Arg Glu Ser Gly Val
    50                  55                  60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser
65                  70                  75                  80

Ile Ser Ser Val Lys Thr Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
                85                  90                  95

Tyr Tyr Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Val Leu
            100                 105                 110

Lys
```

INFORMATION FOR SEQ ID NO:14:

SEQUENCE CHARACTERISTICS:
LENGTH: 113
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: HuCC49 VL
LOCATION: 1..113

DESCRIPTION OF SEQ ID NO:14:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
                5                   10                  15

Asp Arg Val Thr Ile Thr Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
            20                  25                  30

Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Thr Pro Gly Lys
        35                  40                  45

Ala Pro Lys Leu Leu Ile Tyr Trp Ala Ser Ala Arg Glu Ser Gly Val
    50                  55                  60

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr
65                  70                  75                  80

Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln
                85                  90                  95

Tyr Tyr Ser Tyr Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile
            100                 105                 110

Thr
```

INFORMATION FOR SEQ ID NO:15:

SEQUENCE CHARACTERISTICS:
LENGTH: 107
TYPE: amino acid
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: REI VL
LOCATION: 1..107

DESCRIPTION OF SEQ ID NO:15:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
                 5                  10                  15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ile Lys Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Glu Ala Ser Asn Leu Gln Ala Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Gln Ser Leu Pro Tyr
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr
            100                 105
```

INFORMATION FOR SEQ ID NO:16:

SEQUENCE CHARACTERISTICS:
LENGTH: 345
TYPE: DNA
STRANDEDNESS: double
TOPOLOGY: linear

FEATURE:
NAME/KEY: Template used to produce HuvH and HuVHA
LOCATION: 1..345

DESCRIPTION OF SEQ ID NO:16:

```
CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA CCT AGC CAG 48

ACC CTG AGC CTG ACC TGC ACC GTG TCT GGC NNN NNN NNN NNN NNN NNN 96

NNN NNN NNN TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT GAG TGG ATT 144

GGA NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN 192
```

NNN NNN NNN NNN NNN NNN NNN NNN GAC ACC AGC AAG AAC CAG TTC AGC 240

CTG AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC TAT NNN NNN 288

NNN NNN NNN NNN NNN NNN NNN NNN TGG GGC CAA GGG TCC TTG GTC ACC 336

GTC TCC TCA 345

INFORMATION FOR SEQ ID NO:17:

SEQUENCE CHARACTERISTICS:
LENGTH: 345
TYPE: DNA
STRANDEDNESS: double
TOPOLOGY: linear

FEATURE:
NAME/KEY: Template used to produce HuVHS and HuVHK
LOCATION: 1..345

DESCRIPTION OF SEQ ID NO:17:

CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA CCT AGC CAG 48

ACC CTG AGC CTG ACC TGC ACC GTG TCT GGC TAC ACC TTC ACT GAC CAT 96

GCA ATT CAC TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT GAG TGG ATT 144

GGA TAT TTT TCT CCC GGA AAT GAT GAT TTT AAA TAC AAT GAG AGG TTC 192

AAG GGG AGA GTG ACA ATG CTG GCA GAC ACC AGC AAG AAC CAG TTC AGC 240

CTG AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC TAT TTC TGT 288

ACA AGA TCC CTG AAT ATG GCC TAC TGG GGC CAA GGG TCC TTG GTC ACC 336

GTC TCC TCA 345

INFORMATION FOR SEQ ID NO:18:

SEQUENCE CHARACTERISTICS:
LENGTH: 17
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 10
LOCATION: 1..17

DESCRIPTION OF SEQ ID NO:18:

CTAAAACGAC GGCCAGT 17

INFORMATION FOR SEQ ID NO:19:

SEQUENCE CHARACTERISTICS:
LENGTH: 16
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 11
LOCATION: 1..16

DESCRIPTION OF SEQ ID NO:19:

AACAGCTATG ACCATG 16

INFORMATION FOR SEQ ID NO:20:

SEQUENCE CHARACTERISTICS:
LENGTH: 22
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 385
LOCATION: 1..22

DESCRIPTION OF SEQ ID NO:20:

GCGGGCCTCT TCGCTATTAC GC 22

INFORMATION FOR SEQ ID NO:21:

SEQUENCE CHARACTERISTICS:
LENGTH: 22
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 391
LOCATION: 1..22

DESCRIPTION OF SEQ ID NO:21:

CTCTCTCAGG GCCAGGCGGT GA 22

INFORMATION FOR SEQ ID NO:22:

SEQUENCE CHARACTERISTICS:
LENGTH: 60
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 1a
LOCATION: 1..60

DESCRIPTION OF SEQ ID NO:22:

GCTGTCTCAC CCAGTGAATT GCATGGTCAG TGAAGGTGTA GCCAGACACG GTGCAGGTCA 60

INFORMATION FOR SEQ ID NO:23:

SEQUENCE CHARACTERISTICS:
LENGTH: 60
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 1b
LOCATION: 1..60

DESCRIPTION OF SEQ ID NO:23:

GCTGTCTCAC CCAGTGAATT GCATGGTCAG TGAAGGTGTA GCCAGAGCGG GTGCAGGTCA 60

INFORMATION FOR SEQ ID NO:24:

SEQUENCE CHARACTERISTICS:
LENGTH: 94
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 2
LOCATION: 1..94

DESCRIPTION OF SEQ ID NO:24:

CTGGTGTCTG CCAGCATTGT CACTCTCCCC TTGAACCTCT CATTGTATTT AAAATCATCA 60

TTTCCGGGAG AAAAATATCC AATCCACTCA AGAC 94

INFORMATION FOR SEQ ID NO:25:

SEQUENCE CHARACTERISTICS:
LENGTH: 60
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 3
LOCATION: 1..60

DESCRIPTION OF SEQ ID NO:25:

GGACCCTTGG CCCCAGTAGG CCATATTCAG GGATCTTGTA CAGAAATAGA CCGCGGTGTC 60

INFORMATION FOR SEQ ID NO:26:

SEQUENCE CHARACTERISTICS:
LENGTH: 39
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 21
LOCATION: 1..39

DESCRIPTION OF SEQ ID NO:26:

GTTCTTCTGA TTACCACTGT ATAAAAGACT TGACTGGAC 39

INFORMATION FOR SEQ ID NO:27:

SEQUENCE CHARACTERISTICS:
LENGTH: 26
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 22
LOCATION: 1..26

DESCRIPTION OF SEQ ID NO:27:

CAGATTCCCT AGCGGATGCC CAGTAG 26

INFORMATION FOR SEQ ID NO:28:

SEQUENCE CHARACTERISTICS:
LENGTH: 78
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 23
LOCATION: 1..78

DESCRIPTION OF SEQ ID NO:28:

TTCTACTCAC GTGTGATTTG CAGCTTGGTC CCTTGGCCGA ACGTGAGGGG ATAGGAATAG 60

TATTGCTGGC AGTAGTAG 78

INFORMATION FOR SEQ ID NO:29:

SEQUENCE CHARACTERISTICS:
LENGTH: 23
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 24
LOCATION: 1..23

DESCRIPTION OF SEQ ID NO:29:

GCTCTGGGTC ATCTGGATGT CGG 23

INFORMATION FOR SEQ ID NO:30:

SEQUENCE CHARACTERISTICS:
LENGTH: 24
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 4
LOCATION: 1..24

DESCRIPTION OF SEQ ID NO:30:

AGACACCAGC AGCAACCAGT TCAG 24

INFORMATION FOR SEQ ID NO:31:

SEQUENCE CHARACTERISTICS:
LENGTH: 24
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 5
LOCATION: 1..24

DESCRIPTION OF SEQ ID NO:31:

GCTGAACTGG TTGCTGCTGG TGTC 24

INFORMATION FOR SEQ ID NO:32:

SEQUENCE CHARACTERISTICS:
LENGTH: 24
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 6
LOCATION: 1..24

DESCRIPTION OF SEQ ID NO:32:

CTGGCAGACA AGAGCAAGAA CCAG 24

INFORMATION FOR SEQ ID NO:33:

SEQUENCE CHARACTERISTICS:
LENGTH: 24
TYPE: DNA
STRANDEDNESS: single
TOPOLOGY: linear

FEATURE:
NAME/KEY: Oligonucleotide 7
LOCATION: 1..24

DESCRIPTION OF SEQ ID NO:33:

TGGTTCTTGC TCTTGTCTGC CAGC 24

WHAT IS CLAIMED IS:

1. A humanized antibody or humanized antibody fragment which specifically binds TAG-72 which is characterized in that said humanized antibody or humanized antibody fragment is derived from a murine antibody that binds TAG-72.

5 2. The humanized antibody or humanized antibody fragment of Claim 1 wherein the CDRs of said humanized antibody or humanized antibody fragment are obtained from said murine antibody, the VH FRs of said humanized antibody or humanized antibody fragment have an amino acid sequence of the NEWM FRs or the humanized CC49 FRs of Figure 1 or 3, and the VL FRs of said humanized antibody or humanized antibody fragment have an
10 amino acid sequence of the REI FRs or the humanized CC49 FRs of Figure 2 or 4.

3. The humanized antibody or humanized antibody fragment of Claim 2 wherein said humanized antibody has an antigen binding affinity for TAG-72 which is at least 10% that of CC49 and said humanized antibody fragment has an amino acid sequence identical to that of a constituent part of said humanized antibody.

15 4. The humanized antibody or humanized antibody fragment of Claim 2 wherein said humanized antibody has an antigen binding affinity for TAG-72 which is at least 25% that of CC49.

5. The humanized antibody or humanized antibody fragment of one of Claims 2-4 wherein said murine antibody is CC49, CC83, CC46, CC92, CC30, or CC11.

20 6. The humanized antibody or humanized antibody fragment of Claim 5 wherein said murine antibody is CC49.

7. The humanized antibody or humanized antibody fragment of Claim 6 wherein said humanized antibody is expressed by ATCC CRL-12209 and said humanized antibody fragment has an amino acid sequence identical to that of a constituent part of the antibody
25 expressed by ATCC CRL-12209.

8. The humanized antibody or humanized antibody fragment of Claim 1 wherein said humanized antibody or humanized antibody fragment has a humanized variable heavy chain sequence of Figure 1 or 3 or a humanized variable light chain sequence of Figure 2 or 4, or has both said humanized variable heavy chain sequence and said humanized variable light
30 chain sequence.

9. A nucleic acid sequence characterized in that a humanized antibody or humanized antibody fragment according to one of Claims 1-8 may be expressed from the nucleic acid sequence.

10. A vector characterized in that a humanized antibody or humanized antibody
5 fragment according to one of Claims 1-8 may be expressed from the vector.

11. The vector according to Claim 10 wherein said vector is a bare nucleic acid segment, a carrier-associated nucleic acid segment, a nucleoprotein, a plasmid, a virus, a viroid, or a transposable element.

12. A composition suitable for the treatment or *in vivo* or *in vitro* detection of cancer
10 characterized in that it comprises, respectively, a therapeutically effective or a diagnostically effective amount of a humanized antibody or humanized antibody fragment according to one of Claims 1-8.

13. The composition of Claim 12 wherein said humanized antibody or humanized antibody fragment is, directly or indirectly, associated with or linked to an effector moiety
15 having therapeutic activity, and the composition is suitable for the treatment of cancer.

14. The composition of Claim 13 wherein said effector moiety is a radionuclide, therapeutic enzyme, anti-cancer drug, cytokine, cytotoxin, or anti-proliferative agent.

15. The composition of Claim 12 wherein said humanized antibody or humanized antibody fragment is, directly or indirectly, associated with or linked to a detectable label, and
20 the composition is suitable for detection of cancer.

16. The composition of Claim 15 wherein the detectable label is a radionuclide or an enzyme.

17. A method for *in vivo* treatment of a mammal having a TAG-72-expressing cancer characterized in that the method is performed by administering to the mammal a
25 therapeutically effective amount of a composition according to one of Claims 12-14.

18. A method of *in vitro* immunodetection of TAG-72-expressing cancer cells characterized in that the method is performed by contacting the cancer cells with a composition according to any one of Claims 12, 15, and 16.

19. The method of Claim 18 wherein the humanized antibodies or humanized
30 antibody fragments of the composition are bound to a solid support.

20. A method of immunodetection of TAG-72-expressing cancer cells in a mammal characterized in that the method is performed by administering to the mammal a diagnostically effective amount of a composition according to one of Claims 12, 15, and 16.

21. The method of Claim 20 wherein said immunodetection is *in vivo* tumor imaging.

5 22. A method of *in vivo* treatment of cancer by (i) intravenously administering a radionuclide-labeled antibody, (ii) thereafter detecting tumor cells using a radionuclide activity probe, and (iii) thereafter removing the detected tumor cells by surgical excision, characterized in that the antibody is a humanized antibody or humanized antibody fragment according to one of Claims 1-8.

10 23. The method of claim 22, wherein the radionuclide is ^{125}I or ^{131}I.

24. A commercial package characterized in that it contains a composition according to any one of Claims 12-16 as active ingredient together with instructions for use thereof to treat or detect cancer wherein the composition is reconstituted prior to said use.

25. The use of a composition as claimed in any one of Claims 12-16 to treat or detect
15 cancer.

26. The use of a humanized antibody or humanized antibody fragment according to any one of Claims 1-8 for the treatment or detection of cancer.

```
                 10          20    A     30          40          50
                 v           v           v           v           v
CC49MuVH    QVQLQQSDAELVKPGASVKISCKASGYTFTDHAIHWVKQNPEQGLEWIGYF
NEWMVH      QVQLQESGPGLVRPSQTLSLTCTVSG          WVRQPPGRGLEWIG
CC49NMVH    QVQLQESGPGLVRPSQTLSLTCTVSGYTFTDHAIHWVRQPPGRGLEWIGYF
                 ^           ^       ↑  ↑          ^            ^
                 10          20         30         40           50

                 60          70    K S   80          90         100
                 v           v           v           v           v
CC49MuVH    SPGNDDFKYNERFKGKATLTADKSSSTAYVQLNSLTSEDSAVYFCTRSLNM
NEWMVH                            DTSKNQFSLRLSSVTAADTAVY
CC49NMVH    SPGNDDFKYNERFKGRVTMLADTSKNQFSLRLSSVTAADTAVYFCTRSLNM
                 ^           ^ ↑         ^           ^   ↑ ↑   ^
                 60          70          80          90        100

               110
                v
CC49MuVH    AYWGQGTSVTVSS
NEWMVH        WGQGSLVTVSS
CC49NMVH    AYWGQGSLVTVSS
                ^
               110
```

FIGURE 1

```
                   10        20        30        40        50
                    v         v         v         v         v
CC49MuVK  DIVMSQSPSSLPVSVGEKVTLSCKSSQSLLYSGNQKNYLAWYQQKPGQSPK
REIVK     DIQLTQSPSSLSASVGDRVTITCKSS         KNYLAWYQQTPGKAPK
CC49REVK  DIQMTQSPSSLSASVGDRVTITCKSSQSLLYSGNQKNYLAWYQQTPGKAPK
                    ^         ^         ^         ^         ^
                   10        20        30        40        50

                   60        70        80        90       100
                    v         v         v         v         v
CC49MuVK  LLIYWASARESGVPDRFTGSGSGTDFTLSISSVKTEDLAVYYCQQYYSYPLT
REIVK     LLIYWA   ESGVPSRFSGSGSGTDYTFTISSLQPEDIATYYC
CC49REVK  LLIYWASARESGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQYYSYPLT
                    ^         ^         ^         ^         ^
                   60        70        80        90       100

                 110
                  v
CC49MuVK  FGAGTKLVLK
REIVK     FGQGTKLQIT
CC49REVK  FGQGTKLQIT
                  ^
                 110
```

FIGURE 2

FIGURE 3

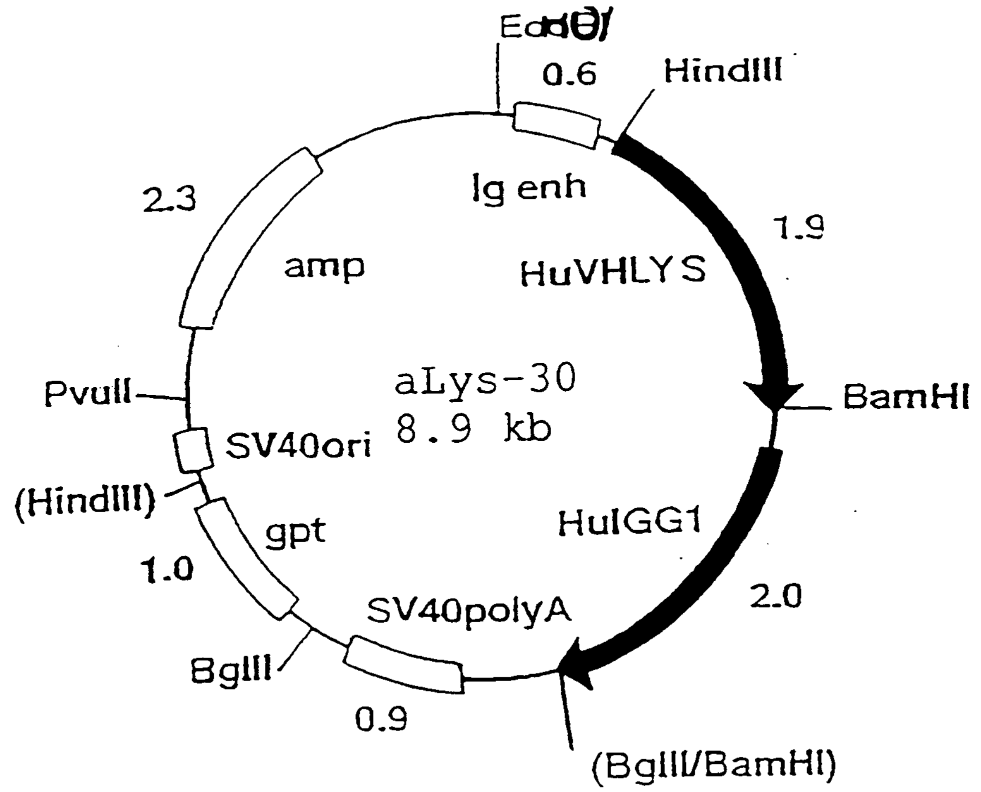
Scotgen Heavy Chain Variable Region Comparison

CC49	Gln	Val	Gln	Leu	Gln	Gln	Ser	Asp	Ala	Glu	Leu	Val	Lys	Pro
HuCC49	Gln	Val	Gln	Leu	Gln	Glu	Ser	Gly	Pro	Gly	Leu	Val	Arg	Pro
NEWM	Gln	Val	Gln	Leu	Glu	Gln	Ser	Gly	Pro	Gly	Leu	Val	Arg	Pro
CC49	Gly	Ala	Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr
HuCC49	Ser	Gln	Thr	Leu	Ser	Leu	Thr	Cys	Thr	Val	Ser	Gly	Tyr	Thr
NEWM	Ser	Gln	Thr	Leu	Ser	Leu	Thr	Cys	Thr	Val	Ser	Gly	Ser	Thr
			CDR1											
CC49	Phe	Thr|	Asp	His	Ala	Ile	His|	Trp	Val	Lys	Gln	Asn	Pro	Glu
HuCC49	Phe	Thr|	Asp	His	Ala	Ile	His|	Trp	Val	Arg	Gln	Pro	Pro	Gly
NEWM	Phe	Ser|	Asn	Asp	Tyr	Tyr	Thr|	Trp	Val	Arg	Gln	Pro	Pro	Gly
													CDR2	
CC49	Gln	Gly	Leu	Glu	Trp	Ile	Gly|	Tyr	Phe	Ser	Pro	Gly	Asn	Asp
HuCC49	Arg	Gly	Leu	Glu	Trp	Ile	Gly|	Tyr	Phe	Ser	Pro	Gly	Asn	Asp
NEWM	Arg	Gly	Leu	Glu	Trp	Ile	Gly|	Tyr	Val	Phe	-	Tyr	His	Gly
CC49	Asp	Phe	Lys	Tyr	Asn	Glu	Arg	Phe	Lys	Gly|	Lys	Ala	Thr	Leu
HuCC49	Asp	Phe	Lys	Tyr	Asn	Glu	Arg	Phe	Lys	Gly|	Arg	Val	Thr	Met
NEWM	Thr	Ser	Asp	Asp	Thr	Thr	Pro	Leu	Arg	Ser|	Arg	Val	Thr	Met
CC49	Thr	Ala	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Val	Gln	Leu	Asn
HuCC49	Leu	Ala	Asp	Thr	Ser	Lys	Asn	Gln	Phe	Ser	Leu	Arg	Leu	Ser
NEWM	Leu	Val	Asp	Thr	Ser	Lys	Asn	Gln	Phe	Ser	Leu	Arg	Leu	Ser
CC49	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Thr	Arg
HuCC49	Ser	Val	Thr	Ala	Ala	Asp	Thr	Ala	Val	Tyr	Phe	Cys	Thr	Arg
NEWM	Ser	Val	Thr	Ala	Ala	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Arg
					CDR3									
CC49	|Ser	Leu	Asn	Met	Ala	Tyr	-	-	-	|Trp	Gly	Gln	Gly	Thr
HuCC49	|Ser	Leu	Asn	Met	Ala	Tyr	-	-	-	|Trp	Gly	Gln	Gly	Ser
NEWM	|Asn	Leu	Ile	Ala	Gly	Cys	Ile	Asp	Val	|Trp	Gly	Gln	Gly	Ser
CC49	Ser	Val	Thr	Val	Ser	Ser								
HuCC49	Leu	Val	Thr	Val	Ser	Ser								
NEWM	Leu	Val	Thr	Val	Ser	Ser								

FIGURE 4

Scotgen Light Chain Variable Region Comparison

CC49	Asp	Ile	Val	Met	Ser	Gln	Ser	Pro	Ser	Ser	Leu	Pro	Val	Ser
HuCC49	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser
REI	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser
				Leu in Figure 2										
CC49	Val	Gly	Glu	Lys	Val	Thr	Leu	Ser	Cys	Lys	Ser	Ser	Gln	Ser
HuCC49	Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Lys	Ser	Ser	Gln	Ser
REI	Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Gln	Ala	Ser	Gln	-
					CDR1									
CC49	Leu	Leu	Tyr	Ser	Gly	Asn	Gln	Lys	Asn	Tyr	Leu	Ala	Trp	Tyr
HuCC49	Leu	Leu	Tyr	Ser	Gly	Asn	Gln	Lys	Asn	Tyr	Leu	Ala	Trp	Tyr
REI	-	-	-	-	-	Asp	Ile	Ile	Lys	Tyr	Leu	Asn	Trp	Tyr
CC49	Gln	Gln	Lys	Pro	Gly	Gln	Ser	Pro	Lys	Leu	Leu	Ile	Tyr	Trp
HuCC49	Gln	Gln	Thr	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Leu	Ile	Tyr	Trp
REI	Gln	Gln	Thr	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Leu	Ile	Tyr	Glu
			CDR2											
CC49	Ala	Ser	Ala	Arg	Glu	Ser	Gly	Val	Pro	Asp	Arg	Phe	Thr	Gly
HuCC49	Ala	Ser	Ala	Arg	Glu	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
REI	Ala	Ser	Asn	Leu	Gln	Ala	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
CC49	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Ser	Ile	Ser	Ser	Val
HuCC49	Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Thr	Phe	Thr	Ile	Ser	Ser	Leu
REI	Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Thr	Phe	Thr	Ile	Ser	Ser	Leu
CC49	Lys	Thr	Glu	Asp	Leu	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Tyr	Tyr
HuCC49	Gln	Pro	Glu	Asp	Ile	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Tyr	Tyr
REI	Gln	Pro	Glu	Asp	Ile	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Tyr	Gln
		CDR3												
CC49	Ser	Tyr	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Val	Leu
HuCC49	Ser	Tyr	Pro	Leu	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Gln	Ile
REI	Ser	Leu	Pro	Tyr	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Gln	Ile
CC49	Lys													
HuCC49	Thr													
REI	Thr													



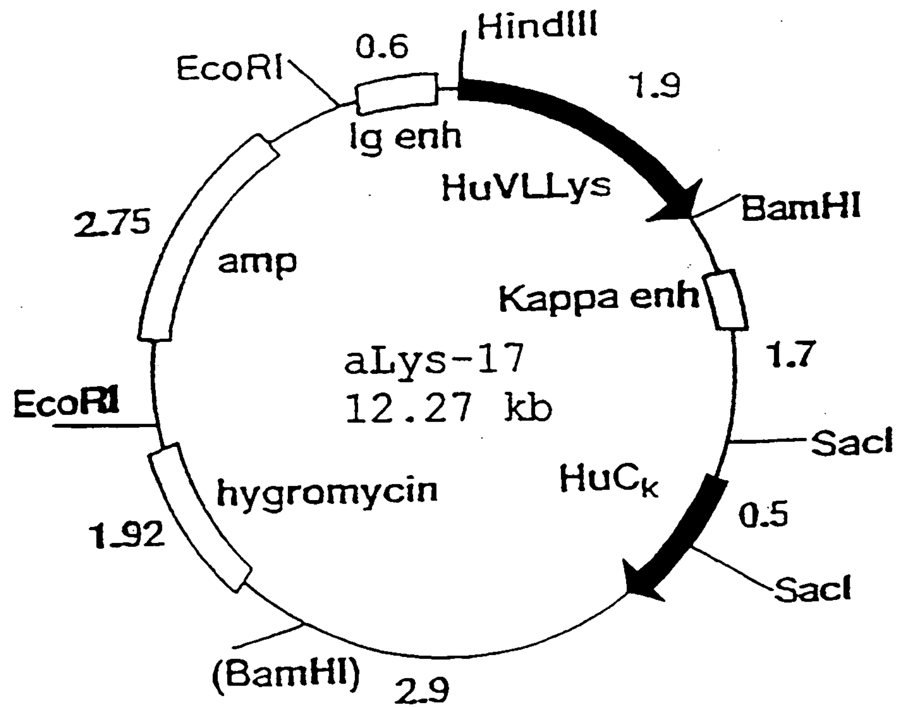
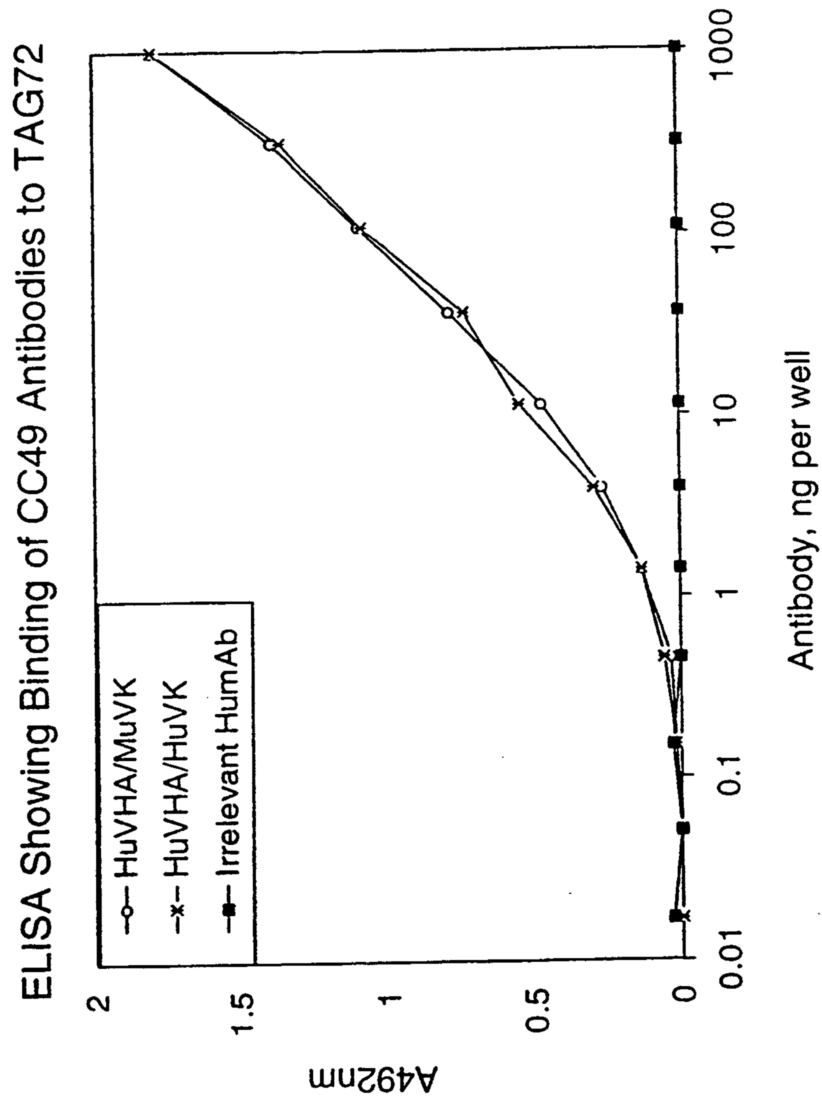


FIG. 5.

Fig. 6



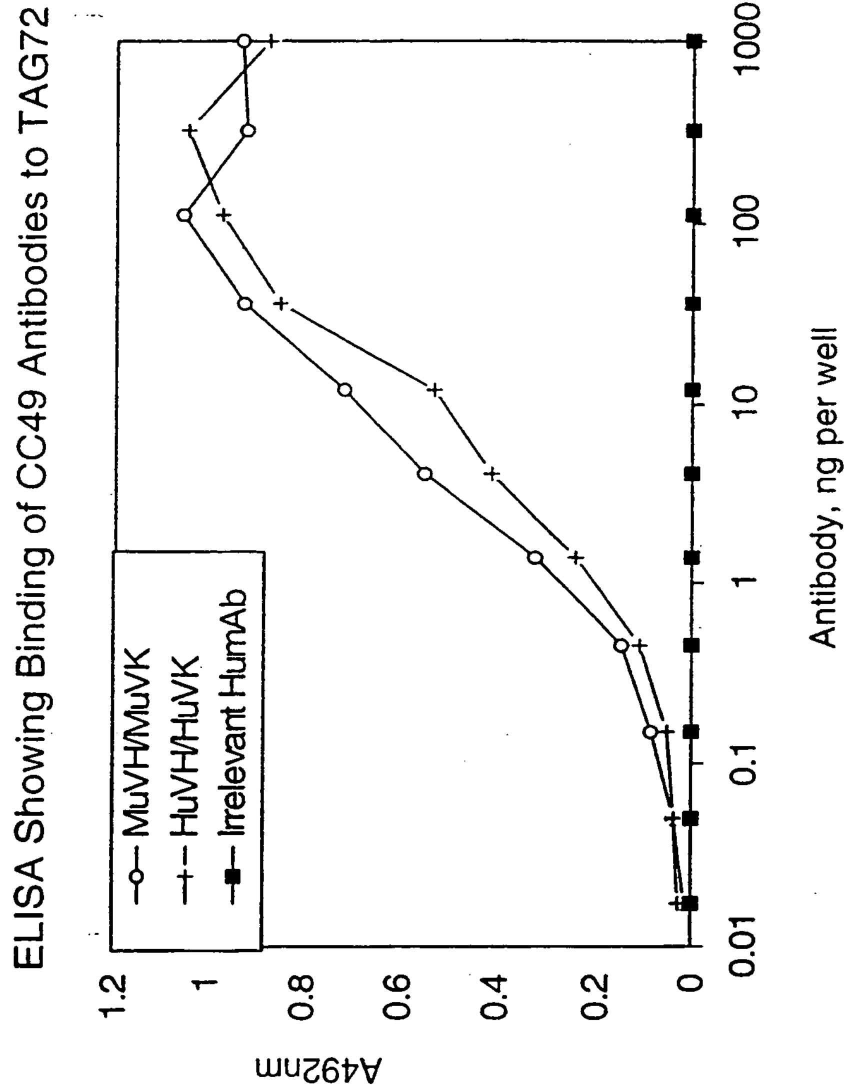
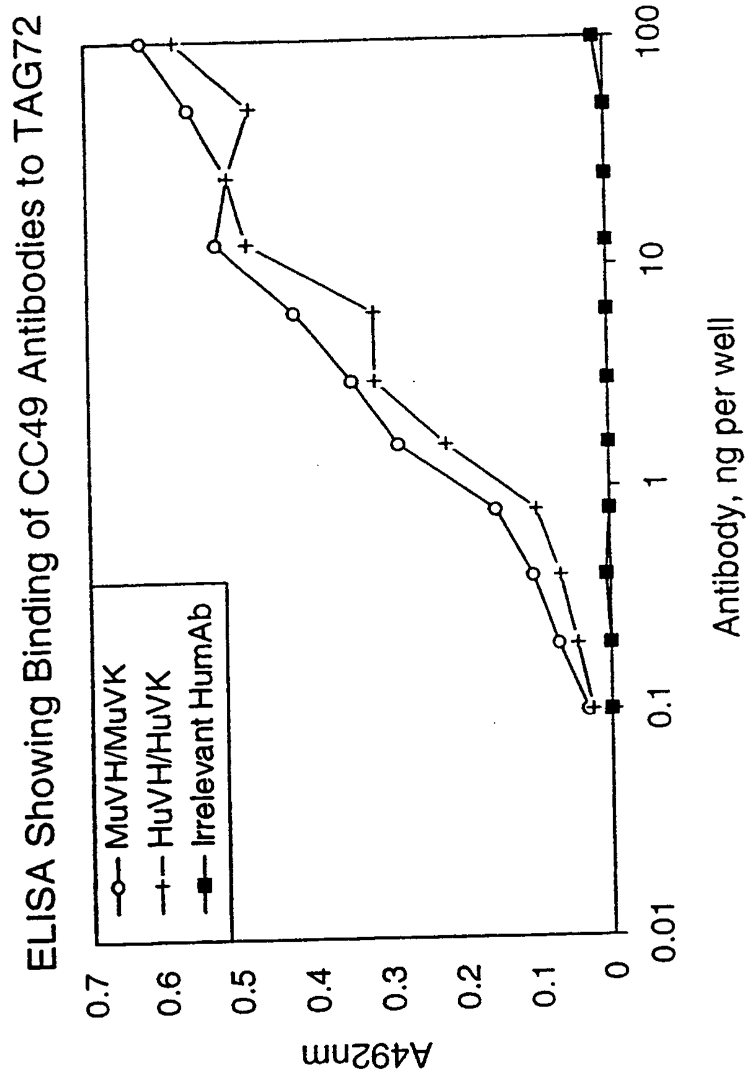


Fig. 7

Fig. 8



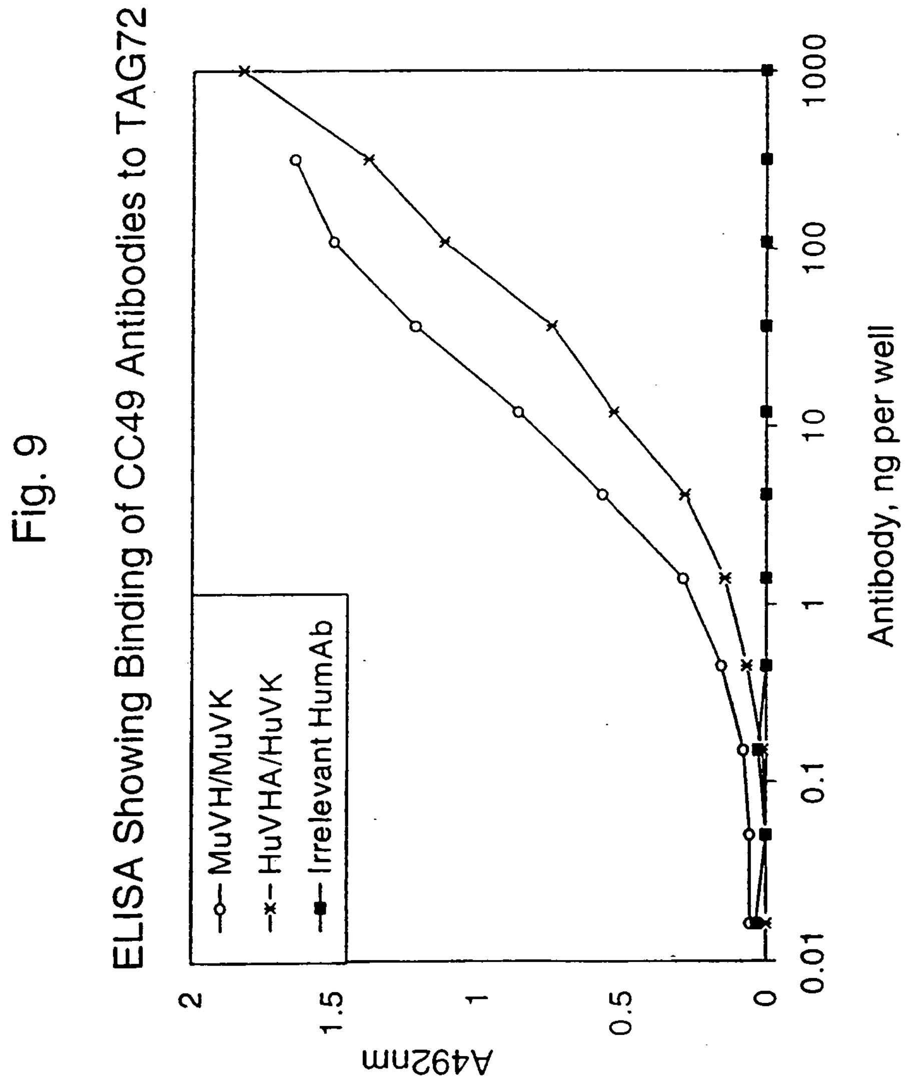


Fig. 9

Fig. 10

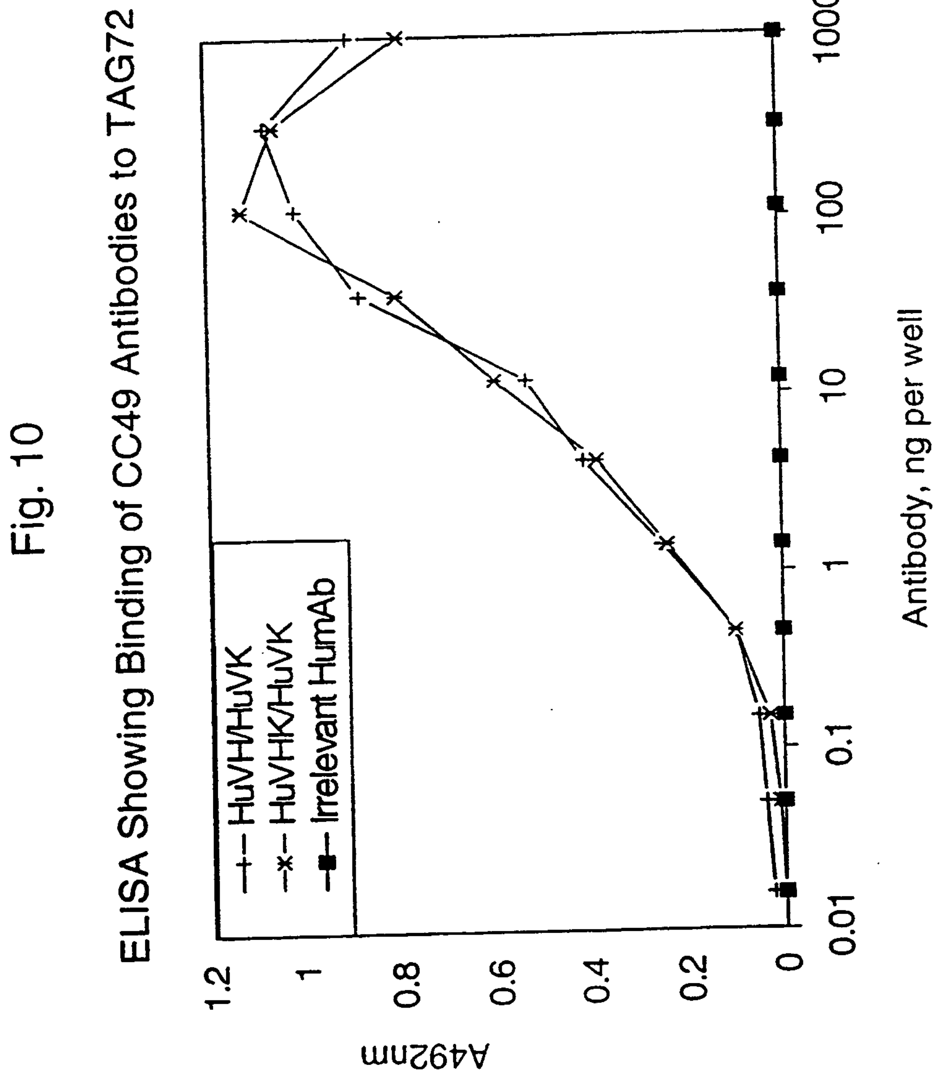
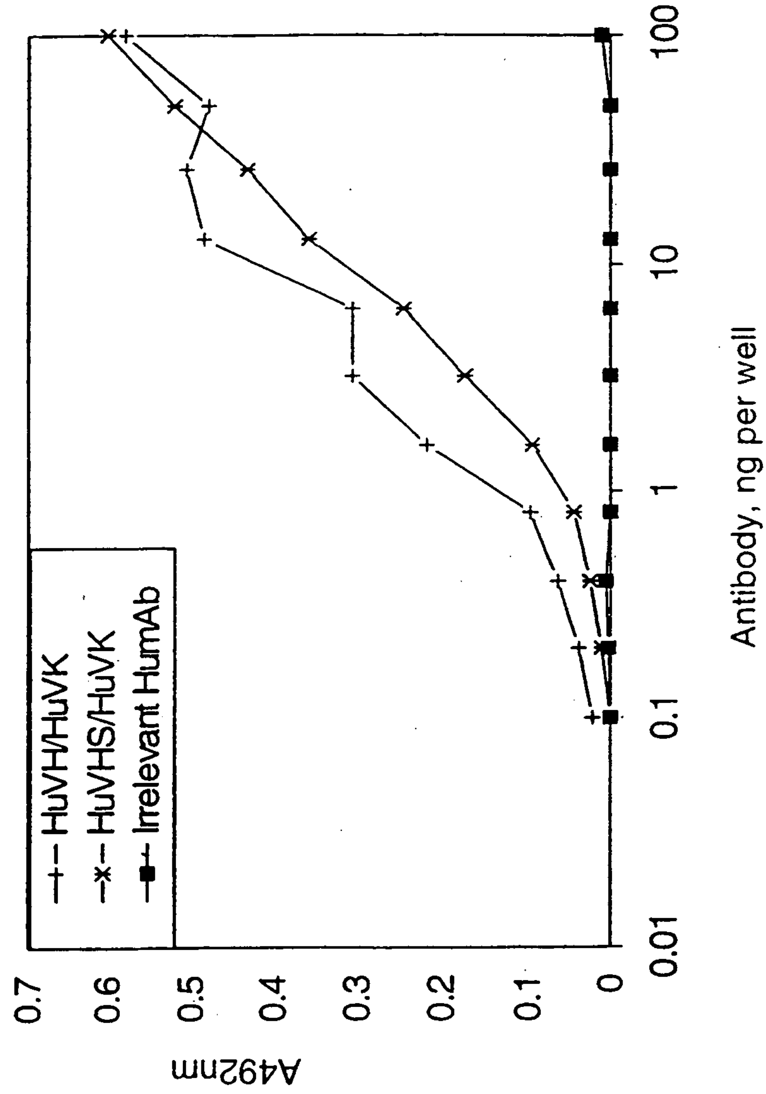


Fig. 11



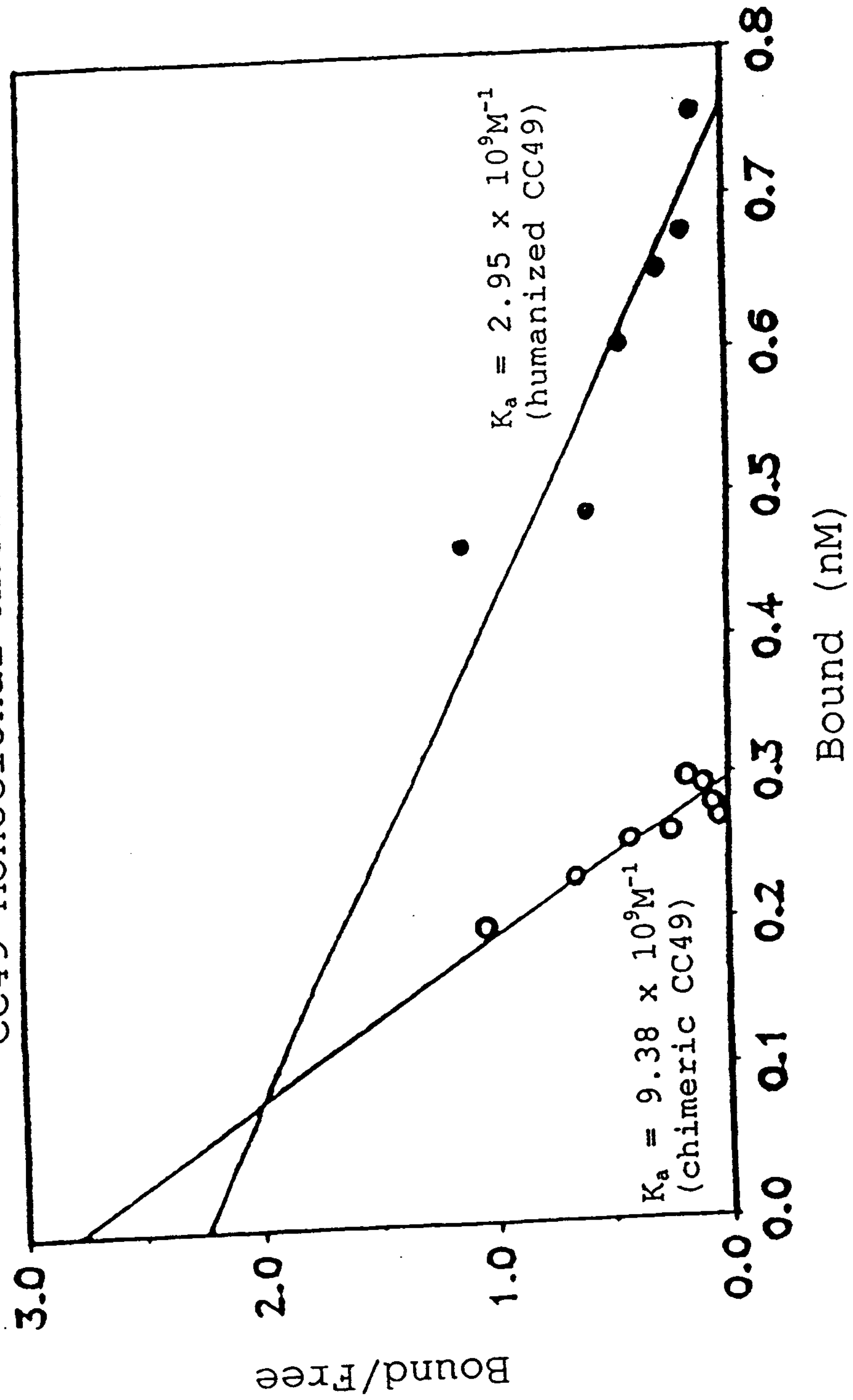


FIGURE 12
Scatchard Analysis of Humanized and Chimerized
CC49 Monoclonal Antibodies
Ka = 2.95 x 10^9 M^-1
(humanized CC49)
Ka = 9.38 x 10^9 M^-1
(chimeric CC49)
Bound/Free
3.0
2.0
1.0
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Bound (nM)

```
              270         280         290         300
               V           V           V           V
CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA CCT AGC CAG

310         320         330                     370
 V           V           V                       V
ACC CTG AGC CTG ACC TGC ACC GTG TCT GGC...   ...TGG GTG AGA CAG

 380         390         400                     480
  V           V           V                       V
CCA CCT GGA CGA GGT CTT GAG TGG ATT GGA...   ...GAC ACC AGC AAG

490         500         510         520         530
 V           V           V           V           V
AAC CAG TTC AGC CTG AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG

   540                  580         590         600
    V                    V           V           V
GTC TAT...   ...TGG GGC CAA GGG TCC TTG GTC ACC GTC TCC TCA
```

FIGURE 13

```
                  270          280          290              300
                   V            V            V                V
CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA CCT AGC CAG
GTC CAG GTT GAC GTC CTC TCG CCA GGT CCA GAA CAC TCT GGA TCG GTC

310             320             330             340             350
 V               V               V               V               V
ACC CTG AGC CTG ACC TGC ACC GTG TCT GGC TAC ACC TTC ACT GAC CAT
TGG GAC TCG GAC TGG ACG TGG CAC AGA CCG ATG TGG AAG TGA CTG GTA

    360             370             380             390             400
     V               V               V               V               V
GCA ATT CAC TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT GAG TGG ATT
CGT TAA GTG ACC CAC TCT GTC GGT GGA CCT GCT CCA GAA CTC ACC TAA

      410             420             430             440             450
       V               V               V               V               V
GGA TAT TTT TCT CCC GGA AAT GAT GAT TTT AAA TAC AAT GAG AGG TTC
CCT ATA AAA AGA GGG CCT TTA CTA CTA AAA TTT ATG TTA CTC TCC AAG

          460             470             480             490             500
           V               V               V               V               V
AAG GGG AGA GTG ACA ATG CTG GCA GAC ACC AGC AAG AAC CAG TTC AGC
TTC CCC TCT CAC TGT TAC GAC CGT CTG TGG TCG TTC TTG GTC AAG TCG

              510             520             530             540
               V               V               V               V
CTG AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC TAT TTC TGT
GAC TCT GAG TCG TCG CAC TGT CGG CGG CTG TGG CGC CAG ATA AAG ACA

550             560             570             580             590
 V               V               V               V               V
ACA AGA TCC CTG AAT ATG GCC TAC TGG GGC CAA GGG TCC TTG GTC ACC
TGT TCT AGG GAC TTA TAC CGG ATG ACC CCG GTT CCC AGG AAC CAG TGG

    600
     V
GTC TCC TCA
CAG AGG AGT
```

FIGURE 14

INTERNATIONAL SEARCH REPORT

Inter: al Application No
PCT/US 98/03679

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/13 C07K16/30 C07K16/46 C12N15/62 C12N15/85
A61K39/395 A61K47/48 A61K51/10 G01N33/574 G01N33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S. KASHMIRI ET AL.: "Generation, characterization, and in vivo studies of humanized anticarcinoma antibody CC49." HYBRIDOMA, vol. 14, no. 5, October 1995, pages 461-473, XP000198397 New York, NY, USA see the whole document ---	1-26
X	D. SLAVIN-CHIORINI ET AL.: "A CDR-grafted (humanized) domain-deleted antitumor antibody." CANCER BIOTHERAPY & RADIOPHARMCEUTICALS, vol. 12, no. 5, October 1997, pages 305-316, XP002079335 New York, NY, USA see abstract see figure 1 ---	1,10-26

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 October 1998	15/10/1998

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/03679

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. MCARTHUR ET AL.: "Targeting of tumor cells by T cells transduced with TAG-72 tumor antigen specific MHC-unrestricted chimeric T cell receptors." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, vol. 38, March 1997, page 37 XP002079336 USA see abstract #244	1,9-13, 17,24-26
X	Y. SHA ET AL.: "A heavy-chain grafted antibody that recognizes the tumor-associated TAG72 antigen." CANCER BIOTHERAPY, vol. 9, no. 4, 1994, pages 341-349, XP002079337 New York, NY, USA see abstract see figure 1	1,9-11
A	EP 0 365 997 A (THE DOW CHEMICAL COMPANY) 2 May 1990 cited in the application see the whole document	1-26

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/03679

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see: Further information continued from PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Although claim 17 (completely) and claims 22, 23, 25 and 26 (all partially) are directed to a method of treatment of the human/animal body, and although claims 20 and 21 (both completely) and claims 22, 23, 25 and 26 (all partially) are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

...ormation on patent family members

Inter al Application No

PCT/US 98/03679

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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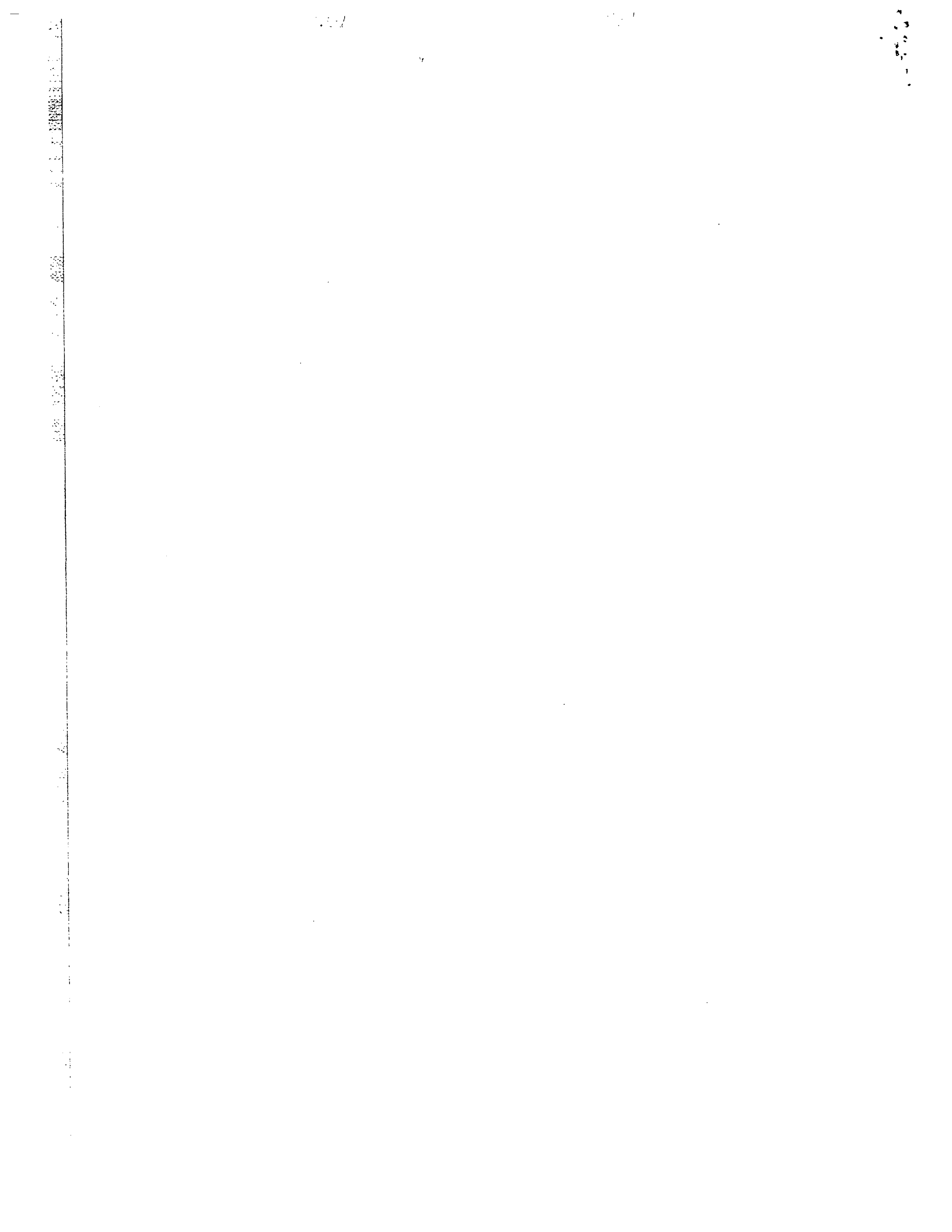

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